AN EVALUATION OF THE ROLE OF RESPIRATORY SYNCYTIAL VIRUS (RSV), ALONE AND WITH OTHER PATHOGENS, IN CAUSING RESPIRATORY DISEASE AMONG NATIVE AMERICAN CHILDREN

by
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Abstract

Respiratory syncytial virus (RSV) is associated with 3.2 million severe lower respiratory illness (LRI) episodes and 118,200 deaths in children <5 years of age annually, with the greatest burden in infants less than 6 months of age. There is no licensed vaccine for RSV, although several candidates are in development. It is not well understood how the prevention of RSV LRI in infancy might impact LRI with other pathogens, or long-term sequelae including subsequent wheeze and asthma. Recently, a double-blinded randomized trial of a monoclonal antibody, motavizumab, showed efficacy for the prevention of RSV-associated medically attended LRI (MALRI) in healthy Native American infants. Infants were followed through three years of age, with nasopharyngeal secretions collected at every MALRI. We tested these samples for respiratory viruses and for *Streptococcus pneumoniae* in order to:

1. Evaluate the role of RSV MALRI prevention in infancy on the prevalence and density of *S. pneumoniae* carriage in the nasopharynx
2. Evaluate the impact of RSV MALRI prevention in infancy on MALRI with other respiratory viruses, and on subsequent medically attended wheeze
3. Evaluate the risk of RSV MALRI in the second year of life

RSV MALRI was associated with increased *S. pneumoniae* density in the nasopharynx, suggesting a role for its prevention in reducing the incidence of pneumococcal pneumonia in vaccinated children and in their communities through indirect protection. Motavizumab prevented RSV MALRI in the presence of co-infecting viruses, with increased efficacy in more severe cases. A family history of asthma, and MALRI in infancy with parainfluenza viruses, rhinovirus and coronaviruses were independently

associated with subsequent medically attended wheeze at ages 1-3 years. Infants who broke through motavizumab prophylaxis to have inpatient RSV LRI had higher risk of medically attended wheezing at ages 1-3 years than children in the placebo group with inpatient RSV LRI, and may represent a subgroup at high risk for both outcomes. We found no increased risk of disease in the second RSV season following receipt of motavizumab in the first season, a finding that has not been demonstrated previously in the context of RSV prevention in healthy infants.

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Abbreviations

AAP	American Academy of Pediatrics
AI	American Indian
AN	Alaskan Native
aOR	Adjusted odds ratio
CAIH	Center for American Indian Health
CFR	Case fatality ratio
CI	Confidence interval
DC	Dendritic cell
EV	Enterovirus
FI	Formalin inactivated
IHS	Indian Health Services
HMPV	Human metapneumovirus
HRV	Human rhinovirus
ICU	Intensive care unit
IL	Interleukin
INF	Interferon
INSPIRE	The Infant Susceptibility to Pulmonary Infections and Asthma following RSV Study
IPD	Invasive pneumococcal disease
IRB	Institutional review board
IRR	Incidence rate ratio
ISAAC	International Study of Asthma and Allergies in Childhood
ITT	Intention to treat
LA	Lung aspirate
LMIC	Low and middle income country
LRI	Lower respiratory illness
mAb	Monoclonal antibody
MALRI	Medically attended lower respiratory illness
NAAT	Nucleic acid amplification test
OM	Otitis media
OR	Odds ratio
PCR	Polymerase chain reaction
PCV	Pneumococcal conjugate vaccine
pDC	Plasmacytoid dendritic cell
PIV	Parainfluenza virus
PRR	Pattern recognition receptor
RR	Relative risk
RSV	Respiratory syncytial virus
SECW	Serious early childhood wheeze
SNP	Single nucleotide polymorphism
TABS	Tennessee Asthma Bronchiolitis Study

TLR	Toll like receptor
UR	Uncertainty range
URI	Upper respiratory illness
WHO	World Health Organization

Chapter 1: Introduction

Respiratory Syncytial Virus (RSV)

Characteristics of the Virus

RSV is a member of the *Paramyxoviridae* family, genus *Pneumovirus*. It is an enveloped, negative-sense single stranded RNA virus, with surface proteins that allow for infection of the human airway epithelial cells. RSV was first isolated from chimpanzees in 1955, and subsequently from infants with lower respiratory tract infection in 1956 [1]. The name 'respiratory syncytial virus' was proposed because infected cells sometimes appeared as large syncytia surrounding multinucleated giant cells [1]. There are two major viral subtypes: RSV-A and RSV-B, each containing clusters of strains with related genotypes [2]. In humans, tropism for the ciliated epithelia of the small bronchioles and type I pneumocytes of the alveoli leads to infection of the respiratory tract, directly causing disease and predisposing the host to superinfections from other pathogens by damaging the epithelium and impeding ciliary clearance of bacteria, as well as via potential synergistic interactions between RSV and other respiratory pathogens [3].

The RSV genome encodes for 11 proteins including nonstructural proteins (NS1, NS2, and M2-2), the (N) viral nucleocapsid protein, the phosphoprotein (P), matrix (M), RNA-dependent polymerase (L), M2-1, and three surface glycoproteins (G [attachment], F [fusion], and SH [small hydrophobic]) [4]. The NS1 and NS2 proteins suppress a key host defense mechanism by blocking signaling of type I and II interferons, and NS2 may

also promote shedding of infected airway epithelial cells [5]. The surface glycoproteins are critical to infection and immune response: the G protein is responsible for viral attachment to the host cell, while the F protein allows fusion between the virion and the host cell and allows entry of the virus into the host [2]. The immune response to infection is mainly targeted to the G and F glycoproteins, the only viral components that induce neutralizing antibody [6]. The F glycoprotein appears to be the most important for generating active immunity, as it induces a high serum antibody response, activation of the pattern recognition receptors CD14 and toll like receptor (TLR)-4, and its genome is highly conserved between RSV groups [4, 6, 7]. The F protein has therefore been the target for most antivirals, prophylaxis strategies, and vaccine candidates [4]. It is a class I fusion glycoprotein that must be cleaved to be activated, and consists of two subunits (F1 and F2) that are linked by disulfide bonds [6]. RSV F is present on the viral surface in both prefusion and postfusion conformations, and when the prefusion form is triggered, it leads to fusion of the viral and cell membranes and changes the conformation to the stable postfusion form [6]. Neutralizing epitopes have been identified on both conformations and monoclonal antibodies directed towards these epitopes prevent fusion of the virus to cell membranes. Site II and site IV epitopes are located on both conformations, and site Ø is located on the prefusion confirmation only. Antibodies directed towards the site Ø are highly neutralizing [6].

Pathogenesis and Clinical Presentation

RSV is considered to be one of the most important causes of acute lower respiratory tract illness (LRI) in infants less than one year of age [8, 9]. Infection in children is almost always symptomatic, with illness ranging from mild upper respiratory illness (URI) to severe and sometimes fatal disease of the lower airways [10].

By the time they reach their second birthday, nearly all children have had an RSV infection, but most experience only a mild URI that does not require medical attention [11, 12]. The incubation period between RSV exposure and symptom onset is typically 4-6 days (range 2-8 days). Common URI symptoms include rhinorrhea, nasal congestion, low-grade intermittent fever and cough [8, 13, 14]. RSV is also the primary virus that invades the middle ear in children with acute otitis media [15]. The acute illness usually lasts 5-10 days with a cough that may persist for several weeks [8].

Involvement of the lower airways occurs in 15-50% of infants and young children with an RSV-infection, typically 1-3 days after the onset of rhinorrhea [8, 10]. RSV-associated LRI (RSV-LRI) is often referred to as "severe RSV illness" and usually corresponds to a clinical diagnosis of bronchiolitis (inflammation and infection of the distal bronchiolar airways, the bronchioles) or pneumonia (inflammation and infection of the alveolar lung regions) [14]. Risk of RSV-associated hospitalization is highest in children less than 3 months of age and typically occurs approximately 4 days after illness onset [16, 17]. In a study of children hospitalized with RSV in the U.S., 85% of those less than 12 months of age and 31% of those 24-59 months of age were diagnosed with bronchiolitis, with

pneumonia and asthma more commonly diagnosed in the older age group (51% and 60%, respectively) [18].

It is presumed that the virus moves from the upper to the lower airways via mechanical aspiration of infectious material, or by directly spreading along the respiratory epithelium [14, 19]. Once in the lower airways, RSV colonizes and replicates within the bronchiolar epithelial cells, causing necrosis of the epithelium [20]. This is consequential for two significant reasons: one is that it depletes the epithelial defense mechanism; the other is that it causes epithelial cells to be sloughed off the wall of the airways and into the lumen of the bronchioles. Bronchospasm may occur as the smooth muscle of the bronchial walls constricts, and mechanical clearance of the sloughed material is reduced due to the damaged cilia [14]. The infection of the lower airways is accompanied by an inflammatory response involving the recruitment of neutrophils, macrophages, lymphocytes and plasma cells [17, 21]. The neutrophils, mucus and other non-cellular material combine with the sloughed epithelial cells to form dense plugs that can occlude the lumen of the bronchioles at the same time that the airway is constricting due to edema. This combination of events is particularly problematic in preterm and very young infants with small diameter airways [3]. Depending on the type of occlusion caused by the plug, airflow both in to and out of the alveolar area may be restricted (bypass valve mechanism), there may be complete obstruction of the airway (stop valve mechanism), air may be allowed out of but not into the alveolar area (ball valve mechanism) or air may be allowed into the alveolar area but not back out (check valve mechanism), with the check valve occlusion resulting in a phenomenon known as gas trapping [20].

Clinical symptoms that distinguish RSV-LRI from URI are the onset of moderate tachypnea, diffuse rhonchi, fine rales and wheezing [14]. In the early stages of lower airway involvement, chest x-rays are normal and the illness may resolve on its own within 1-2 weeks [14]. The precise factors that cause RSV bronchiolitis to spontaneously resolve or to develop into more severe disease are not yet well understood [14]. There is evidence that higher viral loads are associated with increased disease severity, but the extent to which viral load and clinical outcomes are correlated is not fully established [21-25]. A number of genetic polymorphisms that impair the immune response of the human host have been identified and are also associated with more severe disease [26]. Wheezing, caused by the increased rate of airflow through restricted bronchioles, is the most prominent clinical feature of RSV-LRI and most severely affects infants less than three months of age, due to their narrow airways [8, 16]. Along with cough, wheeze worsens as disease becomes more severe [14]. Poor feeding, irritability, and lethargy are also often observed in infants with RSV-LRI [16]. With gas trapping, severe tachypnea and chest hyperexpansion can occur [14]. When the trapped air is re-absorbed, partial or complete collapse of the lung may follow, leading to ventilation perfusion mismatch and hypoxia [14]. Direct infection of the alveolar epithelium or spillover of inflammation from the bronchioles to the alveoli results in RSV-pneumonia, reducing alveolar gas exchange and causing hypoxia [14]. On radiology, partial or complete collapse of the lung may be seen and, less commonly, there may be scattered interstitial infiltrates [8]. Radiological evidence of gas trapping and bronchial thickening may also be present [14]. Hypoxia can lead to apnea and respiratory failure in infants and death may result if

proper care, such as mechanical ventilation, is not received [9]. Pneumothorax and sepsis are also strongly associated with respiratory failure and death in RSV-infected infants [27]. Underlying co-morbidities associated with increased disease severity and risk of death include congenital heart disease, chronic lung disease, and the presence of an immunocompromised state [28]. The case fatality ratio (CFR) for infants hospitalized with RSV is highly dependent on access to care. In infants 6-11 months old in industrialized countries, it is extremely low – with point estimates around 0.1% - while in the lowest income settings point estimates as high as 9.3% in this age group; in younger infants (0-6 months), CFR point estimates range from 0.0% in industrialized countries to 2.7% in low-middle income countries [29]. There is considerable uncertainty in CFR estimates from developing country settings, and RSV deaths may be underestimated as a result of not taking into account the indirect effect of RSV infection on risk of death from other pathogens, such as *Streptococcus pneumoniae.*

Seasonal epidemics of RSV suggest that the development of immunity to the virus prevents continued transmission, but unlike many other viruses, RSV infection does not result in sustained natural immunity to subsequent infections or disease. Reinfection throughout childhood and into adulthood is common, although infection in adults is frequently asymptomatic [1, 8, 30]. The incomplete immunity that results from natural RSV infection is thought to be the result of 'selective immunological amnesia' induced by the virus, though the mechanisms for this are not fully understood [31]. In healthy adults, RSV infection almost always manifests as a common cold, but incidence of severe disease peaks again among the elderly where it is often exacerbated by the presence of

other conditions such as congestive heart failure and chronic obstructive pulmonary disease [8].

The Immune Response to RSV Infection

There are many gaps in our understanding of infant immunology and the immune response to RSV, including the relative contributions of the virus and the host immune response to disease pathogenesis [32]. Some evidence points to a positive relationship between viral load (measured by plaque assay) and disease severity, but this correlation is not fully established [31, 33]. Other evidence suggests that an excessive and dysregulated immune response to RSV infection can be pathogenic and lead to disease-enhancing inflammation [31]. It may be that a sufficiently high viral load is required to establish LRI, but that the severity of illness depends on the subsequent immune response, which is driven by host factors including genetics, age, the respiratory microbiome, and underlying medical conditions [31].

Both humoral and cell-mediated responses are involved in the immune response to RSV. In general, secretory antibodies protect against infection of the upper respiratory tract, while serum antibodies (acquired as a result of natural infection, or received passively through placental transfer or injection of antibodies) protect against infection of the lower respiratory tract, and cell-mediated responses appear to terminate infection [6]. The initial immune response to RSV infection of the ciliated epithelial cells is mediated by pattern recognition receptors (PRR), which are important for recognizing invading pathogens and initiating both the innate and adaptive immune responses [17]. Toll-like

receptors (TLRs) are an important class of PRRs in the RSV response and promote up-regulation of cytokines, chemokines and anti-viral factors [34]. Eosinophils and monocytes are then recruited into the lungs by chemokines. The F-protein of RSV activates TLR-4, likely through interaction with CD14+, a surface molecule expressed on monocytes [21]. TLR-4 causes the release of respiratory inflammatory cytokines interleukin (IL)-6, IL-8, and IL-1β [21]. Neutrophils, induced by IL-8, are the predominant inflammatory cell of the lungs during RSV infection. It is unclear whether significant neutrophilia is pathogenic or protective, as neutrophils release enzymes that damage infected as well as uninfected host tissue [32]. Severe bronchiolitis in infants is associated with an abundant influx of neutrophils that result in mucus overproduction and increased epithelial cell damage [17]. Plasmacytoid dendritic cells (pDC) also play a key role in the innate immune response to RSV by releasing type-1 interferons (INF) to promote viral clearance [17].

RSV infection in young infants is characterized by a poorly protective innate immune response with reduced signaling of TLRs, altered antigen presenting cell function, and enhanced production of pro-inflammatory cytokines [32]. In the presence of RSV, there is also reduced production of antiviral cytokines, such as interferons [32]. The increased susceptibility of young infants to RSV disease may be due to underdeveloped TLR-mediated responses, reduced capacity of their pDC to transport and present antigen and to release type-1 INF, and ineffective natural killer cells, which are necessary for the production of INF-γ that helps drive a robust Th1 response [17, 32].

In the adaptive immune response to RSV, conventional DCs serve as antigen presenting cells and T cells are essential for viral clearance and for virus-specific immune memory [31]. In infants, there is reduced activation of regulatory $CD4^+$ T-cells, which can skew T-cell activation from a protective Th1 response to a pathogenic Th2/Th17 response that is associated with severe disease [32]. There is also impaired T helper cell activation, little or no B cell memory, and inhibition of antibody production by interferon gamma, resulting in low-titer, low-affinity antibody [17, 32].

Although the adaptive immune response is limited in early infancy, it is well documented that IgG antibody, which is directed to RSV G and prefusion RSV F proteins, is received from the mother via placental transfer and colostrum and is present in nearly all infants at birth [35-37]. Maternal antibody titers are inversely associated with both incidence and severity of RSV illness in young infants but quickly decline to very low levels within the first three months of life, corresponding to the peak age of RSV-LRI risk [1]. While young infants can generate their own antibodies in response to RSV infection, the response is impaired in the presence of maternal antibody and generally of poor functional quality. Achieving effective antibody titers by vaccination in young infants is challenging both due to interference by maternal antibody as well as the weak response due to the immaturity of the infant immune system [32].

Several features that have not yet been fully elucidated allow RSV to evade the immune system [31]. These immunomodulatory mechanisms lead to poor immune memory and insufficiently protective RSV-specific serum antibody in both children and adults that

allows for re-infection throughout the lifetime [31]. Longitudinal studies of children over successive RSV seasons have shown that there is limited protection for approximately 6 months following the primary infection [38], although they can be re-infected with the same viral strain within a single RSV season [39], and that the duration of viral shedding is reduced in subsequent infections [40].

Other Viruses Associated with Viral Bronchiolitis

Viral bronchiolitis is estimated to occur in 20-30% of children in the first year of life [41, 42]. RSV and human rhinovirus (HRV) are the most commonly observed etiologies, although bronchiolitis can also be caused by human metapneumovirus, influenza, adenovirus and parainfluenza viruses [43, 44]. RSV is the most frequent etiologic agent, detected in 60-80% of children hospitalized with bronchiolitis [18, 45, 46]. In temperate climates, RSV and HRV have alternating seasonality, with RSV predominating in the winter months (November –April) and HRV peaking in the autumn and spring months [43, 47]. Despite their alternating epidemic peaks, however, RSV and HRV are frequently observed as co-infections because HRV circulates at a high prevalence even in its non-peak months [16, 48]. Compared to other viruses, RSV bronchiolitis is associated with more severe and prolonged disease and higher rates of hospitalization in young children [14]. One reason for this may be the increased cytopathology that RSV exhibits compared to other viruses associated with bronchiolitis [14]. Co-infection by other viruses at the time of RSV infection has not been found to increase severity of illness compared to RSV monoinfection [49].

Diagnosis and Treatment of Acute Illness

Because management and treatment of bronchiolitis is not pathogen-specific, the American Academy of Pediatrics (AAP) recommends diagnosis based on history and physical examination alone, and that laboratory and radiologic studies should not be routinely ordered for diagnostic purposes [28]. For this reason, RSV is often presumed to be the etiological agent of disease during the RSV season and is not confirmed by laboratory testing [14]. For surveillance and clinical trials, however, laboratory confirmation is needed. Available diagnostic tests include culture-based approaches, rapid antigen detection, and nucleic acid amplification tests (NAATs) [16]. Culture tests are sensitive but require a viable virus sample. Point-of-care rapid tests are also available and useful in individuals with higher viral loads (most often infants and children). NAATs are the current laboratory diagnostic of choice for research purposes, with high sensitivity allowing for the detection of virus even in the presence of low viral load. Furthermore, the quantitative capacities of real time PCR tests can provide estimates of viral load that correlate well with viral culture data [16]. NAAT analyses of frozen specimens have shown little difference when compared to fresh aliquots of the same specimen, allowing specimens to be shipped to centralized locations for specialized testing [16, 50]. One of the general disadvantages of NAATs is that their increased sensitivity can result in virus detection in asymptomatic individuals and therefore the presence of the pathogen does not always attribute disease causality. Detection of RSV in the absence of clinical symptoms is rare in infants, occurring in 9% of those regularly tested by PCR surveillance [30], and allowing for etiologic attribution in cases [50].

In addition to the assay used, specimen type contributes significantly to the sensitivity of the diagnostic method. Potential specimen types for RSV diagnosis include nasopharyngeal aspirates and washes, as well as nasopharyngeal, mid-turbinate, nasal or oropharyngeal swabs [51]. Specimens taken from the nasopharynx (including nasopharyngeal aspirates, washes and swabs) exhibit the highest sensitivity for detection of the virus. Nasal (as opposed to nasopharyngeal) swabs are more comfortable for the child and easier to collect, but have been demonstrated to have a lower sensitivity for RSV detection, even when NAATs are used [52, 53]. However, their logistic advantages may justify their use in some contexts, such as community-based surveillance settings.

In industrialized country settings, approximately 1% of patients who seek medical care for RSV-infection will require hospitalization [54]. Even among such settings, management of bronchiolitis varies by country and within country varies by facility and health care provider [28]. The primary treatment is supportive in nature and includes continuous monitoring, intravenous hydration, and supplemental oxygen [54]. The routine use of bronchodilators and corticosteroid medications is not recommended as studies do not show a clear benefit on the overall course of the illness [28]. Ribavirin, a broad-spectrum antiviral, may be considered for use in children with co-morbidities that put them at highest risk of severe disease but is also not recommended for routine use [28]. Antibiotics may be administered if a bacterial co-infection is suspected. On average in the United States, young children hospitalized with RSV-illness are discharged 3.5 days following admission after receiving supportive therapy such as supplemental oxygen and intravenous fluids [14]. In low and middle income countries (LMICs) where most

RSV-associated deaths occur, survival can be improved by increased access to medical care and better availability of pulse oximetry and supplemental oxygen [29].

Risk Factors for RSV-associated Severe Lower Respiratory Illness and Death

Risk factors for severe RSV-LRI

Most children who experience severe illness with RSV are otherwise healthy and have no identifiable risk factors other than young age [10]. The identification of socio-behavioral and biological risk factors associated with the progression of RSV infection to severe illness has therefore been an area of intense research interest.

A meta-analysis of putative risk factors from 20 studies found prematurity (<37 weeks gestation), low birth weight (<2.5 kg), male sex, the presence of siblings, maternal smoking, family history of asthma/atopy, lack of breastfeeding, and living in crowded conditions (≥7 person/household) to be associated with increased risk of RSV-LRI in children less than five years of age [55]. No overall association was found for low parental education, passive smoking, daycare attendance, or indoor air pollution exposure though these have been identified as risk factors in individual studies. There were insufficient numbers of high quality studies to conduct meta-analyses of multiple births, HIV infection in the mother or infant, malnutrition, altitude, previous illness or lack of indoor plumbing as risk factors for severe RSV-LRI [55]. Incomplete immunization, vitamin A deficiency, vitamin D deficiency, zinc deficiency, anemia, birth order and birth interval have been shown to be associated with all-cause ALRI but need further evaluation as risk factors for RSV-LRI [55]. A role for undernutrition as a risk factor for

severe RSV illness is supported by evidence that poor growth (measured as weight-for-age) in infants is associated with increased risk of RSV hospitalization [56].

RSV hospitalizations occur most frequently in infants between 2-3 months of age, and there are several explanations for the elevated risk in this age group [57]. Young infants are at highest risk for severe RSV illness due to their immature immune systems, waning trans-placental maternal antibodies, and narrow airways that are more susceptible to physical obstruction (mean bronchiole diameter in 2-4 month old infants is 120 μm, compared to 250 μm in an adult) [14]. Additionally, young infants have not yet developed lung structure that allows for collateral ventilation, whereby air from healthy alveolar regions can bypass the normal airways through channels and passages to ventilate areas of obstructed airflow [20]. Male infants are at a slightly increased risk for severe RSV-LRI compared to female infants due to shorter and narrower airways that put them at an elevated risk for bronchial obstruction [58]. In temperate climates, birth during the first half of the RSV season is associated with a higher risk of severe disease, potentially due to lower circulating maternal antibodies at the beginning of the RSV season as well as a longer exposure window for infants born early in the season [58, 59].

Preterm birth has been shown to be an independent risk factor for RSV-hospitalization in U.S. population-based surveillance studies [18]. Preterm infants have lower maternal anti-RSV IgG antibodies at birth, as well as increased risk of lower airway obstruction and reduced capacity for gas exchange in the lung compared to term infants [1, 60]. The definition of preterm birth varies by study and setting, generally ranging from <33 to <38

weeks gestation, with increased prematurity (<33 weeks gestation at birth compared to <37 weeks) associated with increased risk of severe RSV-LRI [55].

In addition to prematurity, children with co-morbidities such as chronic lung disease, cyanotic or complicated congenital heart disease and immunosuppression are at higher risk of developing RSV than the general population due to their increased sensitivity to subtle changes in oxygenation, ventilation or pulmonary pressure [21, 61, 62]. Approximately 30% of infants with chronic lung disease of prematurity (CLD) and hemodynamically significant congenital heart disease (CHD) will be hospitalized for an RSV-related illness within the first two years of life, and will experience longer hospitalization stays, a greater need for intensive care, and a higher risk of mortality during their RSV-illness compared to otherwise healthy infants [1]. Congenital abnormalities of the airways, cystic fibrosis, and severe neuromuscular disorders are other co-morbidities that can increase the risk of severe RSV-illness [1]. Additionally, hospital-based studies in South Africa have reported significant associations between HIV-infection and incidence of RSV-LRI [63-65]. Deficiencies in T cell response due to HIV-infection are thought to explain the greater risk of severe disease in this subgroup [32].

Household crowding and day care attendance are consistent environmental risk factors for severe RSV-LRI in infants across several studies, with crowding showing the stronger association with disease status [60, 66]. Crowding is presumed to increase risk of RSV transmission and infection due to increased exposure to droplets of infectious secretions

and fomites [67, 68]. A systematic review of 13 studies found crowding to be associated with laboratory-confirmed RSV illness in both high risk and mixed risk/general population groups, regardless of differences in crowding definitions, study design, or geographic locations [60]. Although different measures of crowding have been used across studies, it is presumed that the most important components of crowding for increased risk of RSV hospitalization are the number of other people living in the home, the proportion of time spent in the crowded residence, and the sharing of bedrooms, although it is challenging to differentiate exposures that happen within the home from those that occur elsewhere [60]. Furthermore, the impact of crowding on risk for severe disease, given infection, may be modified by other environmental factors believed to increase risk of severe respiratory disease such as home construction, the presence of indoor air pollution or tobacco smoke within the home, and climate or altitude (discussed further in Transmission, Circulation and Seasonality section below) [60].

Studies of twins estimate that approximately 20% of overall susceptibility to developing RSV-LRI is attributed to genetic factors [69, 70]. Several single nucleotide polymorphisms (SNPs) of innate immune genes are associated with severe RSV illness, although further research is required to determine whether these mutations themselves are causally associated with severe disease or whether they are associated with other causative variants [26, 32, 69, 71]. Genetic risk factors can also be complicated by environmental interactions. For example, substitutions have been identified in the TLR-4 genome that cause hyporesponsiveness to bacterial endotoxin (LPS) [33, 72, 73]. In a prospective study of infants with RSV-bronchiolitis, it was observed that prior

environmental exposure to LPS activated the TLR-4 response in infants and conditioned the immune response to infection. Infants with the combination of the hyporesponsive TLR-4 phenotype and low exposure to LPS (which corresponded to higher socioeconomic status) had significantly increased risk of severe RSV-LRI after adjusting for all other risk factors [33]. This suggests that prior TLR-4 / environment interactions are important drivers of the immune response to infection and that full term infants with the hyporesponsive phenotype and low LPS exposure may represent a distinct risk group for severe RSV bronchiolitis [33].

Characterization of the respiratory microbiome composition as a risk factor for severe RSV-illness is an emerging area of research. RSV-infected infants with nasopharyngeal microbiota profiles dominated by *Haemophilus influenzae* and *Streptococcus* species have been shown to mount distinct immune responses such as increased TLR-signaling and heightened neutrophil recruitment compared to infants with microbiomes dominated by *Staphylococcus aureus*, and were more likely to experience severe disease [74]. These findings, along with other studies, support the hypothesis that microbial 'cosignaling' could be involved in the inflammatory response to RSV-infection [74].

Risk Factors for RSV-associated mortality

Most studies of RSV-associated mortality have been conducted in middle to upper-income settings, where risk for death is significantly elevated in children with co-morbidities such as preterm birth (weighted mean CFR 1.1%), bronchopulmonary dysplasia (weighted mean CFR 3.1%), and congenital heart disease (weighted mean CFR

5.3%) [75]. A retrospective analysis of globally representative RSV-associated deaths (the RSV-GOLD study) was recently undertaken to better understand risk factors for RSV mortality in LMICs [76]. The analysis showed that children who die from RSV-associated deaths in LMICs tend to have different clinical profiles compared to upper income countries. In lower income countries, children who experience RSV-associated mortality tend to be younger (5 months of age at death, on average, compared to 7 months of age in upper income countries). Co-morbidities (most commonly congenital heart disease, but also HIV-infection, and active tuberculosis) and chromosomal or genetic disorders are present in approximately 28% of children who die in LMICs compared to 70% of children in upper income settings [76]. In all settings, the age at death tended to be higher for children with comorbidities compared to the age of death in otherwise healthy children, including otherwise healthy-preterm children [76]. Some hypothesized reasons for these differences are that deaths of young but otherwise healthy infants in low / lower-middle income countries are reflective of low access to care, and that children born in such settings with severe prematurity or significant comorbidities at birth are more likely to have died in the neonatal period with a different cause of death [76]. In a prospective cohort study in a low-resource area of Argentina, medical complications including sepsis and clinically significant pneumothorax were strongly associated with RSV-respiratory failure and would presumably lead to death in the context of inaccessible or inadequate medical care [27].

Importantly, in low resource settings, a substantial fraction of deaths occur in the community, outside of the hospital setting [77]. Data on RSV-deaths occurring in the

community are limited, in part because post-mortem sample collection is extremely challenging. Because children who have access to care may differ in significant ways from those who do not, it is possible that the clinical picture of children who die in communities is distinct from those who die at facilities. Generating accurate mortality burden estimates in the community is an area of increased research focus [78].

Transmission, Circulation, and Seasonality

RSV transmission occurs by (1) large particle droplet aerosols spread by sneezing, coughing, and breathing, and (2) by infectious secretions contaminating environmental surfaces, which are then self-inoculated [8]. RSV can persist for several hours as a fomite, making it a major hazard for nosocomial respiratory illness on pediatric wards [79]. In addition to respiratory secretions, RSV RNA has also been detected in stool, sweat, and saliva, though it is unknown whether those virions are infectious or constitute a potential mode of transmission [80]. The mean duration of viral shedding, defined as RSV PCR-positive nasopharyngeal samples, is 13.5 days in symptomatic individuals [81]. Young age, severity of illness and the co-occurrence of other viruses during the RSV infection is associated with increased duration of shedding, which in turn increases the probability of transmission [81]. The basic reproductive number (R_0) for RSV, defined as the average number of individuals infected by a typically infectious person in a susceptible population, has been calculated to be 2.18 (95% CI 1.49, 3.24) [82]. In transmission modeling studies in Kenya, household transmission has been found to be responsible for 39% of infant RSV infections, with school-aged children playing a significant role in bringing the virus to the household [82].

RSV has a complex circulation pattern, with the predominant strain changing from year to year and varying within communities [2, 83]. In general, RSV subgroup A is understood to be more virulent and is more often the predominant circulating strain compared to subtype B [2, 84-86]. Unlike rhinovirus and influenza, the same RSV strain can re-infect an individual multiple times [2, 87].

RSV seasonality varies substantially by geography and is marked by seasonal epidemics. Survival of the virus in temperate climates is highest when humidity is <30%, which is often the case during the winter months [8]. The low humidity and temperatures during this season may increase the survival of RSV in the environment, such as on surfaces [88]. Increased crowding and lower micronutrient levels (such as vitamin D) in hosts likely augment survival and transmission of the virus during this time [88]. In such settings, most RSV infection occurs in yearly outbreaks lasting 3-4 months, often in late fall, winter, and early spring (between November and April in the northern hemisphere and March to October in the Southern Hemisphere) [8, 89]. The timing of outbreaks varies by year and within the same year can vary by region and community [8]. In the U.S., surveillance has established that the onset of the RSV season occurs between November and January in most communities, although it has been noted to occur earlier in the year in the southern and eastern U.S. (particularly Florida), and later in the northern and western parts of the country, likely due to differences in climate between these regions [8, 90].

In subtropical regions, RSV peak months typically manifest during the cooler seasons and in tropical/equatorial regions, can occur throughout the year with episodic peaks of higher incidence [91]. Unlike in temperate climates, RSV in tropical regions is usually, but not always, associated with the rainy season [92]. The high humidity during that time may facilitate indirect virus transmission by increasing the amount of time it is encased in droplets on surfaces – once dry, the higher humidity and temperatures tend to decrease virus survival [88]. Similar to winter in temperate regions, transmission during tropical rainy season may be facilitated by increased crowding, reduced sunshine and poorer nutritional status [92]. Seasonal patterns of malnutrition that do not always coincide with the rainy season have been shown to drive RSV seasonality in The Gambia [92]. Seasonal changes in nutritional status may drive RSV transmission patterns in other locations as well, and could explain RSV seasons that occur outside of the rainy season in other tropical settings, such as Nigeria and Kenya [88].

Case Definitions and Endpoints for Surveillance and Clinical Trials

Improvements in surveillance are needed to better define the burden of RSV-illness and strengthen the evidence base that informs vaccine development and policy. It is particularly important to generate disease burden estimates within finer infant age strata, among pregnant women, and in LMIC community settings [93, 94]. With adapted case definitions and surveillance methods, an ongoing effort that could be leveraged for the purpose of RSV surveillance is the Global Influenza Surveillance and Response System (GISRS), a WHO-coordinated network of 143 national influenza centers across 113

member states from all six WHO regions that collects specimens from individuals with respiratory illness [95]. A pilot study is being established to assess the feasibility of using these influenza centers for the purpose of RSV surveillance and will include both hospital and community-based surveillance to establish appropriate case-definitions for RSV-illness in both of these settings [95]. Unlike influenza, children with RSV-LRI are often afebrile at the time of clinic presentation, and surveillance efforts will need to take this distinction into account since the influenza surveillance case definitions include the requirement of fever [96]. Additionally, RSV can present as apnea alone, particularly in very young infants, and this is not captured in the influenza surveillance case definitions [97]. The RSV Global Epidemiology Network (RSV GEN), a collaboration of more than 70 investigator groups, primarily in LMICs, is also working to improve RSV surveillance efforts and data from the investigators forming this collaboration are the basis for updated global RSV disease burden estimates [29].

Medically attended acute lower respiratory infection (MALRI) has been used as a clinical endpoint for RSV immunoprophylaxis studies in industrialized country settings and includes physician visits, urgent care, emergency visits and hospitalization [16]. Expert consensus recommends that in middle and high-income countries, clinical endpoints to measure incidence of severe RSV-LRI should focus on RSV-associated inpatient and outpatient health-care utilization, length of intensive care stay, and reduction in subsequent wheezing and asthma, with RSV infection confirmed if the virus is detected in respiratory specimens within 72 hours of the LRI diagnosis [98]. Because these endpoints are based on physician diagnosis rather than case-defining clinical criteria, they

may be too subjective and dependent on cultural care-seeking norms to compare between different settings [16]. An alternative approach may be to develop illness-based endpoints such as RSV-LRI and RSV-hypoxemia with distinct case definitions based on standardized signs and symptoms, rather than facility-based endpoints [99]. It will be important to ensure that individual measures of clinical signs such as respiratory rate, pulse oximetry and wheezing are collected in a standardized way so that outcome comparisons can be made across studies, even in the context of varying case definitions [100].

Global Burden of RSV

RSV disease burden in children has been defined in many high-income settings, but our understanding of the burden of RSV morbidity and mortality in LMICs is less clear [101]. This data gap is important to address as widespread use of *Haemophilus influenzae* type B (Hib) vaccine and pneumococcal conjugate vaccine (PCV) have reduced the burden of disease from these two pathogens and as a result the proportion of the remaining LRI cases and deaths due to RSV has increased [101]. The true global burden of RSV is challenging to fully quantify not only due to limited surveillance data from developing countries, but also because of difficulty in capturing RSV illness that is not medically attended, and a lack of data regarding laboratory-confirmed RSV-associated deaths [102]. While RSV is not as frequently lethal as bacterial-associated LRI (i.e. the case fatality ratio for RSV is lower than for bacterial disease), its high prevalence makes it quantitatively an important cause of death globally, and accurate estimates of severe

RSV-LRI and RSV-associated deaths are critical for the development of vaccination strategies and policies [27].

The first global burden of disease estimates for RSV were generated for the year 2005 by a systematic review and meta-analysis of published and unpublished data [77]. While these estimates contributed significantly to the field, they were limited by scarce data that were not stratified by narrow age bands, as well as non-uniform case-definitions [29, 101]. Disease burden estimates were updated for 2015 using data from 291 additional studies [29]. With these updated data, it was estimated that RSV is associated with 28% of all child LRI and 13-22% of all child LRI deaths globally [29]. The case definitions for RSV-LRI were as follows: RSV-associated LRI was defined as cough or difficulty breathing with increased respiratory rate for age (consistent with the WHO integrated management of childhood illnesses case definition) and laboratory confirmed RSV; severe RSV-LRI was defined cough or difficulty in breathing with chest wall indrawing and laboratory confirmed RSV (in children less than 2 months of age, an increased respiratory rate >60 breaths/min with RSV confirmation would also meet the case definition of severe RSV LRI, regardless of whether lower chest wall indrawing was present). The addition of a danger sign (cyanosis, difficulty in breastfeeding or drinking, vomiting everything, convulsions, lethargy or unconsciousness, or head-nodding) defined a child as having very severe LRI [29]. Of note, some studies included in the meta-analysis used more restrictive case-definitions, and the sensitivity and specificity of laboratory diagnostics between studies also varied [29]. Approximately 33.1 (uncertainty range [UR] 21.6 – 50.3) million RSV-LRI cases were estimated to occur globally in

2015, with more than 90% occurring in LMICs, and approximately half of the burden borne by five countries (India, China, Pakistan, Indonesia and Nigeria). One fifth of cases were estimated to be severe, defined by the presence of lower chest wall indrawing.

Hospital-associated RSV-LRI was estimated separately, using a case definition of physician confirmed LRI diagnosis with RSV detected from a respiratory sample and a recommendation for hospitalization. Comparing RSV-hospitalization rates alone across countries does not fully reflect the difference in RSV disease burden, because of differences in access to care and care-seeking behaviors contribute substantially to the likelihood of admission. In 2015, there were 3.2 million RSV hospitalizations (UR 2.7 – 3.8 million), with 45% of hospitalizations in children < 6 months, 85% associated with lower chest wall in-drawing, and 20% estimated to be hypoxemic [29]. Hospital case fatality ratios (hCFR) were estimated and were highest among neonates (5.3 deaths/100 cases (95% CI 2.8-9.8 deaths/100 cases) in developing countries), and in low-income countries (9.3 deaths/100 cases [95% CI 3.0 – 28.7 deaths/100 cases] for infants 6-11 months old in these settings) [29]. Overall in-hospital mortality was estimated to be 59,600 deaths (UR 48,000 – 74,500) [29]. Infants less than 6 months of age made up just under half of the burden, with 1.4 million (UR 1.2-1.7 million) estimated hospitalizations and 27,300 (20,700 – 36,200) in-hospital deaths [29]. Using data from three LMIC field sites, out-of hospital fatalities were modeled and added to the in-hospital deaths, resulting in a total estimate of 118,200 (UR 94,600 – 149,400) RSV-associated deaths [29]. Overall, there is a trend over time for decreasing hCFR for RSV-LRI across all regions

and age groups, though 99% of RSV-related mortality still occurs in developing countries [29].

Challenges associated with global RSV disease burden estimation efforts include paucity of data, especially in important regions of the world, few eligible studies, and missing data from existing studies. The greatest uncertainty lies in estimations of RSV-associated deaths, particularly in the first month of life [101]. Due to limited care seeking and the possibility that study hospitals are better resourced than non-study hospitals, it is possible that RSV-associated mortality is underestimated, especially since it may predispose children to bacterial pneumonia [46]. Better estimates of RSV deaths in the community are also critically important. The Child Health Mortality Prevention Surveillance (CHAMPS) study, a 20-year project in six high-mortality settings, will attempt to pair surveillance of community child mortality with post-mortem sample collection to address this data gap [78]. Even in facilities, it is not always possible to collect laboratory samples before death and CFR has been observed to be higher in those who do not have samples collected. While almost all RSV-associated deaths in high-income countries occur in the first year of life, in developing countries RSV related deaths also occur among children who are in their second year of life [46].

RSV Burden in the United States

Population-based surveillance of medically attended RSV-illness in children <5 years of age in the United States has found RSV to be associated with 18% of all respiratory illness, 20% of hospitalizations, 18% of emergency department visits, and 15% of

pediatric office visits during the RSV season (November – April) [18]. It estimated to be associated with approximately 70% of all bronchiolitis and 50% of all pneumonia hospitalizations during the winter season [18, 103]. RSV is estimated to hospitalize 1-2% of the U.S. general infant (<12 mo) population each winter [104]. Although young infants are at the highest risk for severe disease, the burden of RSV-illness, particularly for outpatient visits, remains high through the first 5 years of life, with 61% of outpatients being 24-59 months old [18].

By age, annual RSV hospitalization rates in the U.S. are: 25.9 per 1000 child-years among infants <1 mo; 17.9 per 1000 child-years among infants <2 mo, 17 per 1000 child-years among infants <6 mo, and 3 per 1000 child-years among children less than 5 years of age [18, 105].

Rates of hospitalization for RSV in the U.S. have been shown to be three times higher than those for influenza or parainfluenza viruses in the same populations; rates of RSV-associated outpatient visits were roughly the same as for influenza in older children but significantly higher for RSV in infants less than 6 months old [18]. RSV is associated with 137 deaths per year in the U.S. in children less than 4 years old, compared to 38 per year from influenza [106].

RSV Burden in North American Indigenous Populations

The infectious disease hospitalization rate for the American Indian / Alaskan Native (AI/AN) population has consistently been higher than that of the general U.S. population,

with a particularly pronounced disparity among infants that is largely due to an increased incidence of LRI [107]. Reasons for the increased risk of infectious disease in these settings may include poverty, household crowding, poor indoor air quality, reduced access to plumbing, and in some settings poor access to Indian Health Services (IHS) facilities [107, 108]. It has been suggested that LRI hospitalization rates in some of these communities may be enhanced by a lower threshold for hospitalization [109], but this has been assessed in the Navajo and White Mountain Apache reservations in the U.S. southwest and did not explain the high RSV hospitalization rates in those populations [110].

In multiple surveillance studies, Alaskan Natives and American Indians have been found to be at increased risk for bronchiolitis and RSV-associated hospitalization, with populations in Alaska and the Southwest United States at particularly elevated risk [109, 111-113]. Prospective, population-level, hospital-based surveillance among Navajo and White Mountain Apache populations in the U.S. over three RSV seasons (1997-2000) have attributed 51.3% admissions for LRI among children less than 2 years old to RSV infection, with seasonal hospitalization rates of 63.6 per 1000 children <2 years old and 91.3 per 1000 children <1 year old [110]. For comparison, in the year 2000, the RSV hospitalization rate for children <1 year of age in the general US population with high-risk comorbidities including chronic lung disease, chronic heart disease, Down syndrome, congenital airway abnormalities, preterm birth, and other rare congenital and metabolic comorbidities was approximately 60 per 1000 children [114]. The increased risk of RSV illness in the Navajo and White Mountain Apache populations is consistent with the

increased incidence of LRI associated with other pathogens including *S. pneumoniae* and *H. influenzae* in the same populations [110].

RSV Immunization Strategies: History and Current Landscape

Currently, RSV is the only major etiologic agent of LRI for which no vaccine is available, though several vaccine candidates are in the development pipeline [95, 115]. The RSV vaccine pipeline is one of the most active of all pathogen focal areas, with two classes of products (subunit and live attenuated or recombinant vaccines) targeted towards four distinct populations (Table 1.1) [116]. The goal of achieving sterilizing immunity by vaccination (whereby infection of the upper respiratory tract is prevented) is unlikely to occur, and is therefore not required of vaccine candidates [99]. As of September 2017, there were more than 15 candidate products targeting passive and active immunization against RSV being evaluated in clinical trials [117]. In the United States, the following research gaps have been identified to address in preparation for RSV vaccine introduction: improving surveillance to generate outpatient and inpatient disease burden estimates (particularly within fine age strata and among pregnant women and high risk groups) and developing a better understanding of the impact of RSV infection on recurrent wheeze and asthma in child hood, maternal health and birth outcomes, and quality of life in the elderly [93]. The contribution of severe RSV illness in infancy to long-term outcomes, including the development of subsequent wheeze and asthma will be important to establish in preparation for vaccine policy decisions and to assist with cost-effectiveness studies [93].

Because the majority of global RSV disease burden occurs in otherwise healthy infants, immunization strategies that can be universally applied to protect infants from severe disease are key to achieving a meaningful reduction in disease burden [100, 118]. The World Health Organization (WHO) has identified infants less than 12 months of age as the priority target population for safe, effective, accessible and affordable vaccines to prevent RSV-associated illness and deaths, with particular focus on LMIC settings where disease burden is highest [119]. Young infants who are at highest risk of disease are a particularly challenging group to target for active immunization because of potential competition from maternal antibody as well as their immature immune systems. A notable vaccine failure in the 1960s, described in the next section, and the difficult balance of achieving both safety and immunogenicity further complicate RSV vaccine research in this age group. Other challenging issues in the development and introduction of RSV vaccines include but are not limited to: imperfect animal models, the capacity of the virus to evade the immune system, varied target populations requiring specific vaccine strategies, gaps in existing disease burden data, and challenges in identifying case definitions for vaccine clinical trials. To effectively prevent the majority of RSV infections in childhood and substantially reduce RSV burden, a multipronged approach is likely needed, with maternal immunization pursued in combination with infant immunization [95]. Because the greatest burden of severe RSV disease and death is in developing countries, special consideration will have to be given to vaccination of these populations, including the development of clinical endpoint case definitions that can be compared across diverse settings, as well as consideration of factors that may affect

vaccine efficacy, such as maternal illness, HIV prevalence, crowding, and indoor air pollution [99].

Table 1.1 RSV immunization strategies and target populations for protection

Immunization strategy	Vaccine type	Target population for protection	Furthest current stage of development[1]	Special considerations
Monoclonal antibodies	n/a	Infants <6 months of age	Licensed	Short half-life, available to high-risk populations only. New generation, long-acting products in clinical trials.
Maternal immunization	Non-replicating (particle-based and subunit)	Infants <6 months of age	Phase 3 clinical trials	Generation of safety data, co-morbidities affecting transplacental antibody transfer
Infant immunization	Replicating (vectored and live attenuated)	Infants <6 months of age, as well as older infants	Phase 2 clinical trials	Safety and avoidance of enhanced disease response; competition with maternal antibody; immature immune systems
Adult immunization	Non-replicating (particle-based and subunit)	Elderly (65+ years)	Phase 3 clinical trials	Waning immune response

[1]As of September, 2017 http://www.path.org/publications/files/CVIA_rsv_snapshot_final_0917r.pdf

History of RSV Immunization

In 1969, a formalin-inactivated (FI) RSV vaccine was developed that failed to protect against wild-type (wt) RSV infection, and also produced a 'vaccine enhanced' exaggerated clinical response at the time of natural infection in children who were RSV naïve prior to vaccination [6, 120]. Data from children who were vaccinated with the FI-RSV vaccine as well as animal models suggest that it produced insufficient levels of serum neutralizing antibodies and did not induced local immunity [6]. The vaccine also primed for Th2-like response rather than a $CD8^+$ cytotoxic T-cell response, so once infected with RSV, the virus could not be readily cleared and caused a direct cytopathic effect in the lower respiratory tract as well as increased inflammation and

bronchoconstriction [6]. Recently it has been discovered that the vaccine caused deficient toll-like receptor (TLR) activation of B cells, resulting in a lack of affinity maturation of the antibodies and the deposition of immune complexes in the lungs of children infected with RSV [121, 122]. As a result of the experience with the FI vaccine, live attenuated vaccines, which are most likely to induce protective levels of neutralizing antibody, CD8 RSV-specific cytotoxic T-cells, and a pattern of CD4 response similar to that induced by wt RSV infection, are being pursued for use in infant populations [3, 6].

Passive immunization of infants by monoclonal antibodies (mAbs)

Palivizumab (licensed in 1998) is a neutralizing mAb against a conserved epitope in the RSV viral fusion (F) surface glycoprotein, and is the currently the only licensed antibody for the prevention of RSV in high-risk infants [3]. Palivizumab is directed against the neutralizing epitope on both the pre- and postfusion forms of the F protein, and blocks conformational change that allows viral entry into the cell. It is delivered by intramuscular injection and due to its constrained half-life, has to be delivered monthly during the RSV seasons. Given the cost of palivizumab and the inconvenience of monthly dosing, it is only recommended for use in high-risk infants (Table 1.2). The same constraints make routine use of palivizumab infeasible for use in LMIC settings, where the cost of mAbs have historically been considered prohibitive [95]. The market authorization for a more affordable, generic version of palivizumab is expected to be available at the end of 2018, but a monthly dosing regimen will remain a significant challenge, particularly in settings with weak health systems [95].

Table 1.2 American Academy of Pediatrics red book committee recommendations for Palivizumab (2014)* [123]

High Risk Population	AAP Recommendation
Premature infants (<29 weeks gestation) who are younger than 12 months at the start of RSV season	Palivizumab prophylaxis during the RSV season in the first year of life (a maximum of 5 monthly doses of 15 mg/kg; qualifying infants born during the RSV season may require fewer doses)
Infants born at <32 weeks gestation with chronic lung disease of prematurity requiring ≥28 days of supplemental oxygen after birth, or certain chronic heart diseases, or airway clearance issues	Same as above
Infants born at <32 weeks gestation with chronic lung of prematurity requiring ≥28 days of supplemental oxygen after birth and who continue to require medical intervention during the 6-month period prior to the second RSV season	Palivizumab prophylaxis during the RSV season in the first and second years of life
Children younger than 24 months who will be profoundly immunocompromised during the RSV season	Palivizumab prophylaxis considered during the RSV season

*Due to the high burden of RSV disease and costs associated with transport from remote locations, a broader use of palivizumab may result in Alaska Native and possibly other selected American Indian populations.

Motavizumab is a next-generation humanized IgG1 monoclonal antibody derived from palivizumab that acts against the same epitope on both the pre-fusion and post-fusion forms of the RSV F protein as paliviuzumab but was designed to have an increased affinity for RSV. Both palivizumab and motavizumab prevent virus-to-cell and cell-to-cell fusion in a dose-dependent manner, the mechanism for which appears to be blocking a step in virus replication after attachment to RSV and before virus transcription [124]. Unlike palivizumab, which has only been shown to reduce RSV replication in the lower respiratory tract of cotton rats, motavizumab has been shown in *in vivo* studies to reduce RSV replication in the upper respiratory tract [125]. Owing to its increased potency and affinity for RSV, motavizumab has shown a greater reduction in medically attended LRI compared to palivizumab but also a three-fold higher rate of non-fatal hypersensitivity

adverse events [126, 127]. Because of these adverse events and subsequent additional studies requested by the US Food and Drug Administration, Medimmune is no longer pursuing licensure of this product.

Current efforts are focused on the development of extended half-life mAbs (with the MEDI8897 product furthest in development) that would allow for just one dose to be given at birth or at the beginning of the RSV season and would protect for an entire, typically 5 month season [128]. Such an intervention could be applied to all infants rather than restricted high-risk populations, and could potentially be used in LMICs. If dosing with such a product were to be RSV season-dependent, improved surveillance data would be necessary to accurately track RSV seasonality across LMIC settings.

Passive immunization of infants by maternal immunization

Successful vaccination of pregnant women against RSV in the second or third trimester of pregnancy would result in trans-placental transfer of antibody and the immediate protection of the neonate at birth and for three or more months thereafter [129]. An advantage of this approach is that it protects against RSV-illness during peak susceptibility and also allows for direct vaccination of the infant to be delayed until a point when their immune systems are better equipped for antibody affinity maturation and more efficient antigen presentation [129]. An RSV F nanoparticle vaccine by Novovax is currently in phase III trials in multiple countries, including LMICs, and is the most advanced candidate vaccine for the prevention of RSV in early infancy [95]. Challenges to maternal vaccination include generation of adequate safety data to address

risks to maternal, fetal and neonatal outcomes, determining the optimum time to vaccinate pregnant women (vaccinating late in pregnancy is likely more immunogenic, but would not offer protection to preterm infants; vaccinating early in pregnancy brings the risk of inadvertently ascribing to the vaccine adverse events that are more common during that period), and assessing the potential of maternal antibodies to suppress or interfere with the infant immune response [129]. In LMICs, there are additional special considerations. While transplacental antibody transfer is very efficient in healthy populations, there is concern that underlying health conditions such as hypergammaglobulinema, HIV-infection, under-nutrition and helminth infections may impair antibody transfer [129, 130]. Further research is being done in this area and maternal immunization strategies in settings where such morbidities are common will need to take them into account. Based on current global disease burden estimates and a relatively high RSV CFR in the neonatal period, it estimated that an extended half-life monoclonal antibody or maternal immunization that offered 6 months of protection with 80% efficacy and high coverage could avert up to 1.1 million RSV hospitalizations and 22,000 in hospital deaths globally [29].

Active immunization of infants

Candidate vaccines for active immunization of infants have not progressed as far through the vaccine development pipeline as passive immunization approaches. There are currently two active infant immunization strategies under development: those using viral vector platforms and live-attenuated vaccines. Two live-vectored vaccines targeting infants are currently in phase I and II clinical trials. Several live attenuated RSV strains

are being developed and are currently being evaluated as intranasal vaccines in phase 1 trials [95]. An advantage of live vaccines, which must be highly attenuated, is that they induce broad humoral and cellular immunity without requiring an adjuvant. They are being pursued as the preferred vaccination type in infants because they are unlikely to cause enhanced disease, and so far have not been associated with this outcome in clinical trials [94]. Live attenuated intranasal vaccines have also been shown to replicate in infants in the presence of maternal antibody [6].

An important consideration for infant vaccination programs will be at what age to vaccinate in order to achieve maximum protection. Assuming that maternal antibodies impede response to vaccine, an age window for vaccination must be determined that minimizes the risk of maternal antibody interference while being delivered early enough to prevent severe illness that occurs in the high-risk period of infancy. Data from transmission modeling studies suggest that the ideal age of infant vaccination may be 5-12 months, and may need to be optimized relative to the timing of the RSV season [131]. As an alternative to vaccinating young infants, models taking different levels of vaccine efficacy, lengths of protection, and varying coverage estimates into account suggest that disease transmission to this age group can be significantly reduced by vaccinating school aged children, who are largely responsible for bringing the virus into households [82]. With a vaccine that offers 6 months protection, such an approach could reduce the incidence of RSV-infection in infants by 35% over 10 years [82].

RSV, Subsequent Wheeze, and Asthma

Measuring Wheeze and Asthma in Children

Asthma, defined as a chronic inflammatory disorder associated with reversible airflow obstruction and bronchial hyper-responsiveness, is one of the most common chronic diseases of childhood globally, and has complex environmental and genetic components [71, 132, 133]. The heritability component is particularly strong, with estimates that 25-95% (typical range 60-70%) of asthma is attributable to genetics [71]. Because young children are frequently unable to comply with traditional lung function tests, their diagnosis is usually based on a series of clinical criteria [134]. Asthma can be particularly challenging to distinguish clinically in this age group, however, because young children often present with overlapping features representing a mix of underlying respiratory disease processes [132, 134]. In developing countries, it is estimated that wheeze and asthma in young children are frequently falsely diagnosed as pneumonia, leading to inappropriate care and underestimates of asthma morbidity [135]. Untreated wheezing may contribute to malnutrition (via impaired breastfeeding) as well as serious bacterial pneumonia infections, putting asthmatic children at increased risk of respiratory mortality in these settings [135].

Wheezing is characterized by a continuous whistling sound caused by the narrowing or obstruction of the airways and has many different causes in young children [134]. The most common causes are bronchiolitis and asthma, but other causes may include congenital anatomical abnormalities, foreign body aspiration, and other pulmonary, immune, cardiac and gastrointestinal disorders [134]. Despite the many potential

etiologies of wheeze in children, standardized questionnaires use reported wheeze as a proxy measurement for asthma because it is a highly sensitive tool to use across global settings with varied diagnostic capabilities [132]. Over the past two decades there have been attempts to use longitudinal cohort studies to develop classification systems to predict the risk of asthma in children based on their wheezing phenotype [134]. One of the first classification systems defines three primary childhood wheezing phenotypes: "transient early wheezing", which occurs in the first three years of life and is unrelated to airway hyper responsiveness, "persistent wheezing" and "late-onset wheezing" – the latter two of which are most commonly associate with aeroallergen sensitization and asthma [136, 137].

The Burden of Childhood Wheeze and Asthma

In Europe and the United States, approximately one third of preschool aged children have experienced wheezing in the past six months, and almost 50% of children have had at least one wheezing episode by the time they are 6 years old [134]. Using standardized questionnaires, the International Study of Asthma and Allergies in Childhood (ISAAC) has estimated the prevalence of wheeze in the past 12 months among 6-7 year olds to be 19.1% in North America, and 11.5% globally, while prevalence of severe asthma in the same age group is 7.1% in North America and 4.9% globally [138]. The prevalence of atopic conditions is lower in low-income than high-income countries (odds ratio (OR) 0.49 (95% CI 0.37, 0.66)), which may reflect differences in microbial exposure between these settings (the 'hygiene hypothesis' [138, 139]). Prevalence of wheeze in Alaskan Native and American Indian populations appears to be consistent with that observed in

the general U.S. population, although data are limited and estimates can be difficult to compare due to varying case definitions and age inclusion criteria (Table 1.3).

Table 1.3 Prevalence of wheeze in early childhood in the United States

Study	Year	Location	N	Age	Wheeze Prevalence	Definition
O'Brien et al. [140]	2015	US, Southwest, Native American Indian reservations	641 (placebo group)	1-3 yrs	26.8% 2.7% 14.9%	Medically attended wheeze (MAW) in the past 12 mo 3 or more MAW in past 12 mo Serious Early Childhood Wheeze (see footnote[1])
Patel et al. [141]	2008	US, Various Regions (Review)	Range: 1,500-128,568	2-18 yrs	17.5 - 26.4%	See footnote[2]
Lai et al. [138]	2009	North American sites, ISAAC[3] study	4,014	6-7 yrs	19.1%	At least 1 parental reported wheeze episode in last 12 mo
Bisgaard and Szefler [142]	2007	Multicountry, phone survey	1,000 households (US)	1-5 years	27% in US, no clear geographic gradient	Parental report of cough, wheeze or breathlessness during the recent 6 winter months, 82% of these sought medical treatment (22%)
Martinez, et al. [143]	1995	Arizona (Tucson Study)	826	2 yrs	19.9%	Parent report of transient early wheeze (wheeze with respiratory infection in first three years of life)
Ball et al. [144]	2000	Arizona (Tucson Study follow up)	875	2-3 yrs	24% vs. 17% for those w high vs. low exposure to other children	Frequent wheeze (>3 wheeze episodes during preceding year, reported by parent)
Garner and Kohen [145]	2001	Canada	National Survey of 9,500 households	0-5 yrs	22.1%	Wheezing or whistling in the chest in past year

[1]3 or more MAW events in 12 months or systemic steroids prescribed for MAW event, or asthma-controller medication for wheezing for >=3 consecutive months or 5 cumulative months within a 12 month period or >=1 hospitalization with MAW

[2]One of the following: ISAAC study, with question "Have you had wheezing and whistling in the chest in the last 12 months?" (Yes/No), ISAAC question, but not an ISAAC study, or current wheezing without a diagnosis of asthma, or physician diagnosed asthma.

[3]International Study of Asthma and Allergies in Childhood

RSV-associated Illness and Subsequent Wheeze / Asthma

The onset, course, and severity of asthma are affected by many factors including infectious and non-infectious exposures. A major area of research concerns the elucidation of the role that severe viral respiratory illness in early life, specifically with HRV and RSV, plays in initiating the onset of childhood asthma.

Through observational studies, it has been estimated that 40-50% of infants requiring hospital admission for bronchiolitis will experience subsequent wheezing episodes in early childhood, and 16-48% of infants hospitalized for RSV-LRI will later be diagnosed with childhood asthma [71, 146]. While the association between early life RSV-LRI and subsequent wheeze/asthma is well-established, it has not been established whether RSV lower respiratory tract infection in early life causes wheeze and asthma or whether such infections and RSV disease manifestations are simply more likely to occur in those children who are predisposed to develop wheeze/asthma as they age [2]. A third possibility is that RSV-LRI is both a marker of predisposition to childhood asthma and as well as a factor in the causal pathway [71]. In short, the question is whether or not RSV lower respiratory tract disease is in the causal pathway for subsequent childhood wheezing and if so, whether prevention of early RSV disease will reduce the incidence of childhood wheeze.

Observed association between severe RSV and subsequent wheeze/ asthma

Retrospective and prospective observational studies over the past decades have demonstrated that RSV-associated LRI in infancy is associated with an increased risk of wheeze and asthma during childhood compared to infants who are not hospitalized with RSV-associated illness [2].

Evidence from retrospective studies

Several retrospective studies have established a specific association between bronchiolitis medical visits or hospitalization (without pathogen specific information) in infancy and subsequent wheeze or asthma [147-149]. The Tennessee Asthma Bronchiolitis Study (TABS) used their population-based birth cohort to retrospectively analyze the relationship between bronchiolitis hospitalization and subsequent asthma. They found that children who had bronchiolitis during the RSV season were more likely to develop asthma between 4 and 5.5 years of age (RR: 1.89; 95% CI: 1.80-2.00) than those who did not have bronchiolitis during RSV season, but they did not use virologic methods to confirm RSV as the cause of the bronchiolitis [150]. Retrospective studies that did virologically confirm RSV-associated severe illness have also found an association with subsequent wheeze or asthma [151-154]. While some of these studies found a high risk of wheeze around age three to four years that subsequently dropped off by age five to six [152] or ten years of age [147], one study found an increased risk of asthma up to age 20 [154].

Evidence from Prospective Studies

In the 1990s, two large prospective cohort studies were conducted that established early life severe RSV illness as an important risk factor for asthma- and/or wheeze-associated illness through adolescence [137, 155]. In a cohort of Swedish infants who were hospitalized with RSV-bronchiolitis and followed through age 18 years, an increased risk of asthma, recurrent wheezing, or allergic sensitization was observed at three, seven, and 18 years of age compared with the control group, and reduced lung function (independent of asthma) was observed through seven years of age [155-158]. Although RSV bronchiolitis in infancy was the most important risk factor for the development of subsequent asthma, family history of asthma/atopy increased the risk as well [158].

In the Tuscon Children's respiratory study, a cohort of U.S. children followed through 13 years of age produced somewhat different findings [137]. In this study, a broader definition of exposure was used (any medically attended RSV-confirmed LRI rather than limiting to hospitalized RSV-bronchiolitis as in the Swedish study), and an association was observed with increased wheeze at age 6 and 11 years, but not at age 13 years of age. The study also reported that RSV-LRI in the first three years of life was associated with transient early wheezing (defined as one lower respiratory tract illness wheezing during the first three years of life, but no wheezing at six years of age), or non-atopic wheezing later in life, but not with IgE-associated wheeze [159]. Observational studies of the RSV-wheeze/asthma relationship from developing countries are fewer. One study from The Gambia found a significantly increased risk of wheeze within the first two years of life following hospital admission for severe RSV disease (IRR 7.33; 95%CI: 3.10,17.54), but the association dropped off by three years of age [160].

Severity of RSV infection in relation to subsequent risk of wheeze or asthma

Most studies of the relationship between RSV infection and wheeze/asthma have focused on medically attended RSV infections, and several use hospitalization to define their exposed groups. Within these studies, relationships between disease severity and increased risk of wheeze have been established using clinical and laboratory markers of severity. In the TABS cohort, more severe bronchiolitis was associated with greater odds of developing childhood asthma [150]. In a study using data from U.S. managed-care organizations, increased risk of wheeze through five years of age was observed in children who had been hospitalized with RSV, compared to those with outpatient visits, though those with outpatient RSV visits still had a slightly elevated risk of wheeze compared to those who had no RSV-associated visit [161]. A retrospective study of full-term, previously healthy infants who were hospitalized for bronchiolitis during the first year of life and subsequently experienced recurrent wheeze (defined as two or more physician-verified episodes of wheezing a year for three consecutive years) found that those who presented with wheeze in the first 36 months of life had higher RSV viral loads during their bronchiolitis event in infancy, compared to wheezing-negative patients [146].

Mechanisms by which RSV-illness may predispose to wheeze and asthma

RSV lower respiratory infection in infancy is hypothesized to predispose children to asthma through several biological mechanisms including chronic epithelial and airway reactivity changes to the developing infant lung; lung injury altering lung function; and

immunomodulatory changes [2]. Because severe RSV disease is associated with Th2 polarization of the infant immune system, it may sensitize the host immune response to other allergens [162], and this response may be further exacerbated in children with a high genetic risk of atopy, who have a heightened deficit in Th1 function [163]. It has also been demonstrated that RSV-illness in early life promotes overexpression of nerve growth factor (NGF), which can cause airway hyperreactivity that drives short and long term changes in the behavior nerves across the pulmonary system [2]. Severe RSV-illness is associated with reduced lung function that persists through childhood; whether or not this causes persistent hyperreactivity and asthma is an area of research [2]. The relationship between viral infection and asthma is further complicated by recent research highlighting a potential interaction with colonizing bacteria of the respiratory tract [164]. In particular, studies of the nasopharyngeal microbiome found that early asymptomatic colonization with *Streptococcus* was a strong risk factor for asthma, and that bacterial co-colonization at the time of upper viral infection during infancy was associated with the spread of virus to the lower airways and subsequent inflammatory responses that may contribute to risk of asthma development [165]. Additional research is needed in this area to better understand the implications of specific bacterial co-infections with RSV and the risk of subsequent wheeze and asthma.

Contrasting evidence comes from animal and clinical studies demonstrating that upper respiratory tract RSV infections may in fact reduce the risk of asthma development, presumably by modulating pathogenic immunity through early activation and expansion of T regulatory cells [166-168]. If this is indeed the case, it has significant implications for RSV vaccine and immunoprophylaxis development [168].

Evidence for shared host factors for both asthma and acute severe bronchiolitis

An important limitation of the observational studies described here is that the children who develop severe RSV illness early in life may be different from those who do not develop severe illness during the same RSV season, and these features themselves may be the causal risk factor for subsequent wheezing. Several studies have found evidence of this phenomenon. In the Copenhagen Prospective Study of Asthma in Childhood birth cohort, increased neonatal bronchial responsiveness was associated with increased risk of medically assessed viral bronchiolitis, including RSV-bronchiolitis [169]. In another cohort of infants, maternal asthma was found to be a risk factor for respiratory infection in infants, independent of subsequent childhood wheezing illness [170]. Decreased lung function at birth, preceding RSV-LRI has also been found to be a risk factor for severe RSV-LRI as well as for subsequent wheeze [171]. Preterm birth has been shown to have a dose-response relationship to the risk of developing asthma in childhood, though a large retrospective study establishing this association did not control for exposure to RSV-LRI [172].

A number of genes that are associated with both increased risk of asthma and RSV-illness have been identified, though most studies focus on single genes and more complex gene-gene and gene-environment relationships may be important for determining the RSV-asthma relationship [2, 71, 173]. Genetic epidemiology shows promise as a means to identify common biologic pathways for asthma and RSV-illness, and to potentially quantify the extent to which these two outcomes share common genetic predispositions

[71]. Compared to asthma studies, there have been very few studies of candidate genes for risk of severe RSV-illness, and so far no genome wide association studies, reducing the number of common genes that can be identified between the two conditions [71]. Studies of genetic risk for severe RSV-illness can be challenging due to environmental factors that influence exposure to RSV infection, such as exposure to RSV season during periods of immune development and peak susceptibility [71].

Evidence from intervention studies

In the absence of a licensed RSV vaccine, immunoprophylaxis intervention studies offer the most promising method for discerning the causal role of RSV-illness on incidence of subsequent wheeze and asthma, but there are relatively few of these (Table 1.4). The intervention studies that have been conducted include a mix of randomized and non-randomized trials among mostly non-representative populations using nonstandard case-definitions, making cross-study comparisons and extrapolation to the general population difficult [174].

In all but one of the intervention studies, a reduction in wheezing outcome was observed among the exposed (prophylaxed) participants (Table 1.4). The only study to assess the impact of RSV immunoprophylaxis on the risk of subsequent medically-attended wheeze in healthy full-term infants found no protective effect of the intervention; those who received motavizumab had a similar risk of subsequent wheeze through 3 years of age compared to placebo recipients [140]. One other double-blind randomized intervention trial has been conducted in preterm infants, and found a 73% relative reduction (95% CI:

66-80%) in total number of wheezing days over the first year of life outside of the RSV season in those who received palivizumab, compared to placebo [175]. Unlike the study conducted in healthy full-term infants, this study used parent-reported wheeze as an outcome, rather than medically attended wheeze, making comparisons between the two studies difficult. It is also possible that the relationship between RSV LRI and subsequent wheeze is different in preterm infants than it is in term infants due to differences in lung and immune system development. The conflicting observations from these intervention studies casts doubt on the assumption that preventing RSV-illness in infancy will necessarily produce a population-level reduction in wheeze and asthma in later childhood. It also highlights the need to develop standardized outcome measures by which to evaluate this relationship. It will be very important for future RSV vaccine trials to measure subsequent wheeze in a standardize manner in order to better understand this relationship [93].

Table 1.4 Study characteristics and wheezing outcomes of respiratory syncytial virus prophylaxis trials and observational studies

	US RSV Intravenous immunoglobulin study [176]	Canada-Europe palivizumab Study [177]		Japan palivizumab study [178]; atopic asthma substudy [179]	Dutch palivizumab study [175]	Native American Infants motavizumab study [140]
Study Characteristics						
Study Type	RCT and Observational	Observational		Observational	RCT	RCT
Blinded	No	No		No	Yes	Yes
Comparison Group	Placebo and Non-treated	Non-treated		Non-treated	Placebo	Placebo
Study Years	1999-2000	2001-2004		2007-10	2008-11	2004 -10
Length of follow-up for wheeze outcome	single observation in each child	24 mo		2-3 yrs; 6 yrs	6-12 mo.	24 mo.
Age range during which wheezing outcome measured	7 – 10 years	2-5 years		4 mo – 3 years; 6 years	0-12 mo	1-3 years
Study population	Preterm ≤35 wGA with BPD/CLD	Preterm ≤35 wGA with no CLD		Preterm 33-35 wGA with no CLD	Preterm 33-35 wGA with no CLD	Healthy term infants ≥36 wGA
Control group (n)	26	230		95	215	641
		100 (No family history of atopy or food allergies)	130 (Family history of atopy or food allergies)			
Respiratory Disease Outcomes (Comparison Group)						
FEV_1/FVC <80%	62% (16/26)					
Recurrent wheeze (parent)					21% (45/215)[a]	
Recurrent wheeze (parent or medically attended)		26% (59/230)[b]				
		Not reported	Not reported			
Recurrent wheeze (medically attended)		16% (37/230)		at 4 mo – 3 years: 19% (18/95)[c]		3% (16/641)
		16% (16/100)	16% (21/130)			
Serious Early Childhood Wheezing[d] (medically attended)						14% (90/641)
Outpatient medically attended RSV-LRI during season of RSV prophylaxis	81% (21/26; not stratified as outpatient or inpatient)				4.7% (10/215)	10% (71/710)[e]
RSV-hospitalization during season of RSV prophylaxis		30%[f] (76/230)			5.1% (11/215)	11% (80/710)[e]
		25% (25/100)[f]	39% (51/130)[f]			
Probable asthma[j]				18.2% (12/66)		

	US RSV Intravenous immunoglobulin study [176]	Canada-Europe palivizumab Study [177]		Japan palivizumab study [178]; atopic asthma substudy [179]	Dutch palivizumab study [175]	Native American Infants motavizumab study [140]
Family history (Comparison group)						
Asthma	≥8% and ≤12% (4% mother; 8% father)	25.7%[b]		23% (29% in subgroup included in asthma substudy)	≥12% and ≤ 23% (12% mother; 11% father)	12%[e]
		0%	45.4%			
Eczema		10.8%[g]			≥15% and ≤29% (15% mother; 14% father)	5%[e]
		0%	19.2%			
Hay fever		30.9%[g]			≥26% and ≤49% (23% mother; 26% father)	9%[e]
		0%	54.6%			
Atopy	≥23% and ≤38% (23% mother; 15% father)	51.3%[g]		70%	≥37% - ≤71% (34% mother; 37% father)	21%[e]
		0%	90.8%			
Households with a smoker		57%	37%	63%	≤44% (17% mother; 27% father)	24%[e]
Maternal smoking during pregnancy	58%				16%	6%[e]
Wheezing outcome (% relative reduction among prophylaxis group versus comparator group)						
Abnormal FEV_1/FVC	75% [1-(15%/62%)][h]					
Recurrent wheeze (parent reported)					47%	
Recurrent wheeze (parent reported or medically attended)		49%[b]				
		Not reported	Not reported			
Recurrent wheeze (medically attended)		51%[b] [RR 0.49 (95%CI: 0.28, 0.86)]		At 4 mo – 3 yrs: 66% [RR 0.34 (95% CI: 0.19, 0.60)] At 6 yrs: 53% [RR 0.47 (95%CI: 0.25, 0.88), no difference in subgroup without family history of allergy/asthma		No difference [RR 1.10 (95% CI: 0.61, 1.97)]
		69% [RR 0.31(95%CI: 0.12, 0.81)][i]	No difference [RR 0.69 (95%CI: 0.34, 1.39)][i]			
Probable asthma				No difference [RR 0.82 (95%CI: 0.39, 1.70)		

[a]Includes wheezing occurring during the first RSV season [175], whereas other studies exclude this period
[b]Obtained from earlier published analysis of entire control cohort [180]
[c]Active medical care seeking for all respiratory symptoms stipulated by protocol [178]
[d]Severe Early Childhood Wheeze (SECW) was defined as medically attended recurrent wheeze, one or more hospitalizations for wheezing, systemic steroids for a medically attended wheezing event, or asthma controller medication for wheezing over a 12 month period for ≥3 consecutive months or 5 cumulative months) [140]

[e]Denominator is entire ITT control cohort, n=710 [140]
[f]Documented hospitalization for RSV in the first year before enrollment. Participants were enrolled if they were ≤36 months of age [177]
[g]Calculated by combining results of the 130 children with family history of atopy presented in Table II with the 100 control children without such history [177]
[h]Inferred from paragraph 2, page 629 [176]
[i]Figure 1, univariate analysis [177]
[j]Recurrent wheezing with a high serum total or specific IgE level or a family history of allergy

Ongoing research on wheeze and asthma following RSV infection

Currently, a population-based longitudinal cohort study (The Infant Susceptibility to Pulmonary Infections and Asthma Following RSV Exposure (INSPIRE) Study) is being conducted in Tennessee to better understand how the spectrum of early RSV infection (from mild to severe) influences the development of wheeze and asthma [168]. Specifically, it will (1) characterize the host phenotypic response to RSV infection in infancy and risk of recurrent wheeze and asthma, (2) identify the immune response and lung injury patterns of RSV infection that are associated with the development of early childhood wheezing and asthma, and (3) determine the contribution of specific RSV strains to early childhood wheezing and asthma development [168]. Nearly 2,000 healthy term infants living in Tennessee have been enrolled over two years and will have surveys conducted every two weeks during November to March in the first year of life, receive in-person visits when criteria are met for respiratory illness, and have the primary study endpoint (wheezing illness) assessed annually up to age 3-4 years. Samples collected will allow baseline and follow-up measurements of the respiratory and gut microbiomes, inflammation, immune response and lung injury, as well as RSV and HRV detection during respiratory illnesses [168]. This study design, however, will be unable to avoid the potential confounding that is inherent in observational studies of the RSV/wheeze/asthma relationship.

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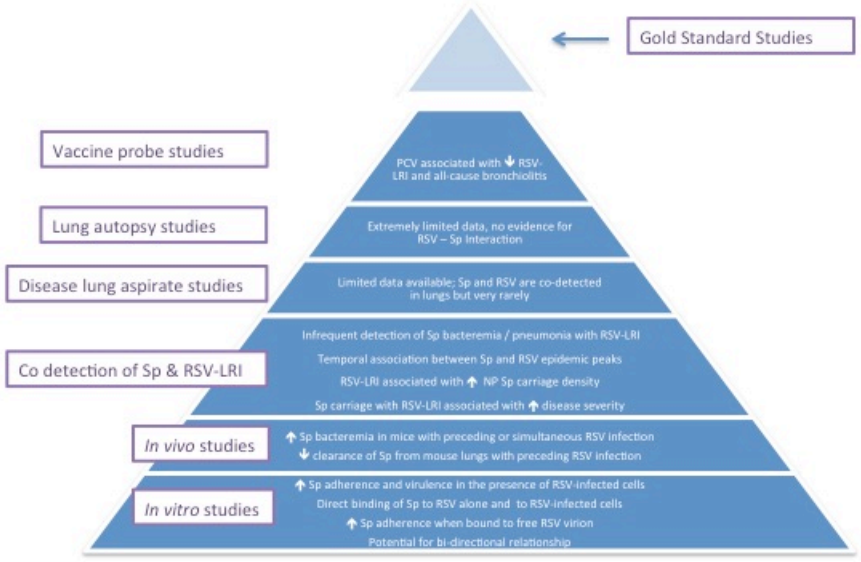
Chapter 2: A review of the interaction between *Streptococcus pneumoniae* and RSV in acute lower respiratory illness

Acute respiratory illness is an important cause of childhood morbidity and mortality globally, with pneumonia accounting for 16% of all under-five deaths in 2015 [181]. In 2015, after more than 130 countries had introduced pneumococcal conjugate vaccine (PCV), there remained an estimated 335,000 deaths from pneumococcal disease among children under five years, accounting for more than one third of total pneumonia deaths [182]. Respiratory syncytial virus (RSV) is another important pathogen, recognized as one of the most common causes of lower respiratory infection (LRI) in children, and particularly among young infants [46]. There is currently no licensed vaccine to prevent RSV, which causes approximately 59,600 - 118,200 RSV-associated deaths in children less than five annually, accounting for 7-13% of pneumonia deaths in this age group [29]. Mixed pathogen infections are common in LRI, including pneumonia, where it is increasingly understood that most cases are a result of sequential or coincident multi-pathogen infections [183-186]. A growing body of evidence suggests that the clinical severity of lower respiratory disease may be increased in the context of such mixed infections [187]. Mechanisms by which viruses can predispose to bacterial infections are well described and include increased bacterial adherence, reduced bacterial clearance in the presence of virus, exacerbated host immunopathology, and suppression of the immune response following viral infection [188, 189]. The best understood example of this type of interaction is the phenomenon of influenza virus predisposing to secondary pneumococcal infection, which was responsible for much, if not most, of the massive

mortality suffered during the 1918 influenza pandemic [190]. Over the past 20 years, a body of evidence has grown describing a complex, multifactorial process whereby bacterial and viral factors, in combination with the host immune response, result in more severe disease with pneumococcus and influenza compared to influenza alone [190]. The evidence for an interaction between RSV and pneumococcus is not as well established as that for influenza and pneumococcus, but there is plausibility for a causal, potentially bi-directional, interaction between these two common pathogens in children with lower respiratory disease. Understanding interactions between RSV and pneumococcus and their role in pediatric LRI may be relevant to the improvement of prevention and treatment strategies, as well as for the for the investment case for RSV vaccines and continued investment case for pneumococcal vaccines. We aimed to collect, evaluate, and synthesize the experimental and epidemiologic data that provides evidence for an interaction between pneumococcus and RSV in causing lower respiratory illness in children, highlighting strengths and identifying where gaps may remain in our understanding of the relationship between these two pathogens (Figure 2.1).

Figure 2.1 Evidence for an interaction between RSV and Streptococcus *pneumoniae* in causing lower respiratory illness



Key:
PCV: pneumococcal conjugate vaccine
RSV-LRI: respiratory syncytial virus-associated lower respiratory illness
Sp: *Streptococcus pneumoniae*
IPD: invasive pneumococcal disease
NP: nasopharynx
↑: increased

Evidence of interaction between RSV and pneumococcus from *in vitro* studies

In vitro studies provide evidence that RSV infection preceding pneumococcal colonization results in enhanced bacterial attachment to host cells and increased bacterial virulence, and that simultaneous rather than sequential infection with the two pathogens may result in even greater propensity for enhanced disease. There is also evidence, albeit limited, of a potentially bidirectional relationship whereby preceding pneumococcal colonization could facilitate RSV infection.

The respiratory epithelium provides the first line of immune defense against invading bacteria, and preceding viral infection can disrupt this layer to allow for enhanced

bacterial infection [187]. Increased bacterial adhesion to virus-infected epithelial cells has been observed for several pathogen combinations and is considered an important step in the process leading to secondary bacterial infection [191]. Pneumococcus and RSV have been shown to interact in this way, with increased adhesion of pneumococcus to RSV-infected epithelial cells demonstrated experimentally *in vitro* [192-196]. Proposed mechanisms for this phenomenon include up-regulation of bacterial receptors in RSV-infected cells, direct binding of pneumococcus to RSV glycoprotein, and increased expression of bacterial neuraminidase in the presence of RSV, discussed below.

RSV glycoprotein G (RSV-G) is a transmembrane viral protein that mediates attachment to host cells and is expressed on the surface of RSV-infected eukaryotic cells [197]. When expressed on epithelial cells, it results in increased transcription and expression of several host bacterial receptors that lead to enhanced adherence by pneumococcus [195, 196, 198]. Consistent with this increase in receptors, pneumococcus in cell cultures forms bacterial aggregates around the ciliary tips of RSV-infected structures where RSV-G proteins are expressed [199]. Pneumococcal adherence is further enhanced by the demonstrated ability of the bacteria to bind to RSV-G protein directly [194, 198]. The binding site for RSV-G has been identified as a protein expressed in the bacterial cell wall (pneumococcal penicillin binding protein 1a (PBP1a)) [199], and no difference in binding to RSV-G has been observed by pneumococcal serotype [194]. In addition, pneumococcus can bind directly to free virions via the RSV-G protein, thereby forming RSV-pneumococcal complexes that infect previously uninfected cells [194, 198]. Adherence of pneumococci to uninfected cells increases dramatically following the

formation of such complexes [194]. Finally, increased expression of the enzyme neuraminidase in the presence of RSV may further facilitate bacterial adhesion [199].

In addition to enhancing bacterial adherence to the epithelium, RSV may promote pneumococcal infection by exacerbating damage to the epithelial cilia and increasing pneumococcal virulence. Pneumococci that have been incubated with RSV or purified RSV-G protein produce more of the chemokine responsible for neutrophil recruitment into the airways and cause greater ciliary damage, leading to reduced bacterial clearance [199]. The binding of RSV-G and PBP1a also results in upregulation of the gene pneumolysin, which encodes a toxin that has been shown to be critical for the development of invasive pneumococcal disease [199].

Additional experimental evidence supports a bidirectional relationship between the two pathogens, whereby preceding or simultaneous pneumococcal exposure may enhance the ability of RSV to infect the host. Bacterial neuraminidases produced by pneumococcus enhance RSV infection *in vitro*, potentially by improving the interaction between RSV transmembrane glycoprotein and its cellular receptor through the removal of sialic acids [200]. Additionally, a synthetic bacterial lipopeptide has been shown to enhance RSV attachment to host cells when simultaneously added to cell culture with RSV (but not when added after virus) [201]. Prior colonization with pneumococcal serotypes 8, 15A or 19F, but not 19A or 23F, has been shown to result in significantly higher numbers of RSV-infected epithelial cells [202]. These results were not fully consistent in cotton rats, however, where serotypes 19F and 23F, but not 8 or 15A, were associated with higher

subsequent viral loads [202]. Because only one strain of each serotype was tested in this study, however, it was not possible to determine whether differences were indeed serotype related [202]. In another study, cells incubated with pneumococcus prior to RSV infection showed no difference in the amount of inflammatory cytokines IL-6 and IL-8 secreted compared to RSV-infected cells that were not pre-exposed to pneumococcus, though the authors speculated that the use of heat-inactivated bacteria could have altered the pneumococcal virulence and may have obscured the results [203].

Evidence of interaction between RSV and pneumococcus from *in vivo* studies

In vivo studies provide strong evidence that RSV and pneumococcal co-infection can lead to increased disease severity compared to infection by either pathogen alone. Infecting mice with RSV and then pneumococcus, or with RSV and pneumococcus together, increased the incidence and level of subsequent bacteremia compared to those infected with pneumococcus alone [194]. The greatest effect was observed with simultaneous infection, supporting the hypothesis that greater bacterial binding occurs in the context of pneumococcal-RSV infecting complexes, and was consistent with another study where simultaneous treatment of mice with RSV and pneumococcus lead to significant disease and rapid death [199]. Similar patterns of enhanced bacteremia have been observed with simultaneous infection of streptococcus and influenza virus [204]. A relationship in the opposite direction, with pneumococcal inoculation preceding RSV infection, was not assessed in these studies.

RSV pre-infection also results in increased risk of pneumococcal pneumonia in mice. In a sequential pulmonary infection mouse model, infection with even very low titers of RSV followed by intratracheal inoculation of pneumococcus resulted in higher bacterial loads and decreased bacterial clearance from the lungs compared to mice without preceding RSV infection [205]. Bacterial clearance was depressed even in the presence of increased macrophages and neutrophils in bronchoalveolar lavage samples, leading the investigators to hypothesize that RSV may have induced functional changes in the recruited neutrophils or other changes in the inflammatory/cytokine milieu in the mouse lung [205]. In this study, a similar effect was observed with RSV infection preceding inoculation with *Staphylococcus aureus* or *Pseudomonas aeruginosa*, was dependent on the dose of virus and bacteria used, and persisted beyond the time that RSV could be detected in the lungs [205]. The effect of pneumococcal infection preceding RSV was not investigated in the mouse pulmonary model.

Finally, *in vivo* models of influenza and pneumococcus have demonstrated immune cell infiltration after dual infection to be greater than by either infection alone, and although it has not yet been specifically demonstrated for RSV and pneumococcus, it is possible that the combination of these two pathogens similarly triggers an overactive immune response and subsequent immunopathogology [204, 206].

Evidence from epidemiologic studies

Pneumococcal bacteremia and pneumonia are detected infrequently in children with RSV-LRI

Co-detection of pneumococcal bacteremia is rare in children with severe RSV-LRI, and occurs most frequently in very young infants or those with underlying health conditions including preterm birth. A number of retrospective case-series studies have attempted to define the proportion of children with severe RSV-LRI who have bacteremia, primarily for the purpose of informing treatment guidelines and identifying children at increased risk of death (Table 2.1). These studies included hospitalized and outpatient cases with RSV-LRI confirmed by antigen testing or antigen testing in combination with viral culture, were for the most part conducted in low mortality settings prior to PCV introduction, and reported positive blood cultures in 0%-2.8% of severe RSV-LRI cases [207-217]. In studies that limited cases to those admitted to intensive care units (ICUs), the proportion blood culture positive rose to 0.6%-3.7% [218-221]. A significant exception to the trend occurred in a high-mortality setting (Pakistan in the 1980s), where more than one third of children <5 with RSV-LRI had bacteremia and 12.4% of children with RSV-LRI had pneumococcus detected by blood culture [216].

Risk factors for bacteremia in children with RSV-LRI have been assessed and include preterm birth, age less than three months, nosocomial RSV, underlying medical conditions and ICU admission [211, 212, 215]. Co-occurrence of severe RSV-LRI and serious bacterial infection (not limited to pneumococcus) was associated with more severe disease, longer hospitalization periods and increased requirements for mechanical ventilation compared to RSV-LRI alone [220, 222].

Although bacteremia is usually a rare complicating event in children with RSV-LRI, pneumococcus makes up a large proportion of the pathogenic blood culture isolates in most studies (Table 2.1). It is possible that these studies may also be underestimating the true prevalence of pneumococcal pneumonia co-infection as a result of antibiotic exposure and low sensitivity of blood culture methods. Bacteremia has a very low sensitivity for detection of true bacterial pneumonia, and studies reporting on bacteremia in children with RSV-LRI should be interpreted with that lack of sensitivity in mind. In children with RSV-LRI for whom bacteremia was defined by positive blood culture or blood PCR the proportion with bacteria detected in blood was higher, with 10% overall bacteria positive, and 3.7% pneumococcal PCR positive [215].

Table 2.1 Pneumococcal bacteremia in children with RSV-LRI[1]

Author, setting, study type	Inclusion criteria	Method for bacterial detection	N	Bacteremia n (%)[2]	Proportion of Sp+[3] bacteremic cases n (%)
Bloomfield. Low mortality, Australia [211] (retrospective)	0-14 yrs inpatient RSV-LRI with blood culture taken within 30 days of RSV(+) result	Blood culture	861	11 (0.6%); higher incidence among those admitted to ICU[4] (2.9%) 6 (0.7%) Sp+	6/11 (56%)
Byington, low mortality, US [223] (prospective)	Febrile infants <90 days w RSV-infection (inpatient and outpatient)	Blood culture	159	0 (0.0%)	n/a
Cebey-Lopez, low mortality [215] (prospective)	<2 yrs inpatient with RSV-LRI	Blood culture Blood PCR	66	1 (1.5%) Sp+ 7 (10%) 3 (4.5%) Sp+	1/1 (100%) 3/7 (43%)
Duttweiler, Low mortality, Switzerland [219] (prospective)	<12 mo, inpatient RSV-LRI admitted to ICU	Blood culture	127	3 (2.4%) 2 (1.6%) Sp+	2/3 (67%)
Ghafoor, high mortality, Pakistan [216] (prospective)	<5 yr, inpatient and outpatient RSV-LRI	Blood culture	491	175 (36%) 61 (12%) Sp+	61/175 (35%)
Hall, low mortality, US [207] (prospective)	<3 yrs, inpatient RSV-LRI	Blood culture	565	3 (0.5%) 1 (1.8%) Sp+	1/3 (33%)
Kneyber, Low mortality, The Netherlands [220] (retrospective)	<12 mo inpatient RSV-LRI, admitted to ICU for respiratory failure or apnea	Blood culture	27[5]	1/27 (3.7%) 0 (0.0%) Sp+	0/1 (0.0%)
Liebelt, Low mortality, US [208] (retrospective)	≤90 days presenting to emergency department with RSV-LRI	Blood culture	120	0 (0.0%)	n/a
Oray-Schrom, Low mortality, US [214] (retrospective)	≤90 days, RSV-LRI presenting to emergency department (80% admitted)	Blood culture	120	1 (0.8%) 0 (0.0%) Sp+	0/1 (0.0%)
Purcell, Low mortality, US [213] (retrospective)	Inpatient infants and children[6] with discharge diagnosis of RSV-LRI	Blood culture	2,396	0 (0.0%)	n/a
Randolph, low mortality, US [221] (retrospective)	Otherwise healthy children <36 mo admitted to ICU with	Blood	165	1 (0.6%) Sp+	1/1 (100%)

Author, setting, study type	Inclusion criteria	Method for bacterial detection	N	Bacteremia n (%)[2]	Proportion of Sp+[3] bacteremic cases n (%)
	confirmed RSV infection; excluded underlying conditions and preterm birth				
Resch, low mortality, Austria [222] (prospective)	<12 mo inpatient RSV-LRI	Blood culture	464	2 (0.4%) 0 (0.0%) Sp+	0/2 (0.0%)
Rice, low mortality, US [217] (prospective)	<2 yrs RSV-LRI with CXR available	Blood culture	38	1 (2.6%) Sp+	1/1 (100%)
Titus and Wright, Low mortality, US [210] (retrospective)	Infants ≤8 weeks RSV pos with fever, admitted to hospital	Blood culture	170	0 (0.0%)	n/a
Timmons, low mortality, [212] (retrospective)	Inpatient infants and children (age not specified) with pos RSV antigen test	Blood culture	108	3 (2.8%) Sp+	n/a (it was not reported whether other bacteria were detected)
Tristram, low mortality, US [224] (retrospective)	Inpatient children ≤5 yrs with confirmed RSV infection, included those w underlying medical conditions	Blood culture	189	4 (2.1%) Sp+	4/4 (100%)

[1]We excluded studies that assessed bacteremia in bronchiolitis cases where RSV infection was not confirmed, as well as studies that reported bacterial outcomes in RSV-LRI cases without specifying the number of pneumococcal-positive cases among those with RSV-LRI
[2]Percentages rounded to first decimal place if <10%; percent all-cause bacteremia unless "Sp+" specified
[3]*Streptococcus pneumoniae* detected
[4]Intensive care unit
[5]n=82 children were included in this study but only 27 had blood cultures collected
[6]Upper age limit for inclusion not specified, but 95.3% of cases were < 2ys old

The proportion of children with pneumococcal pneumonia, rather than bacteremia, in the presence of RSV-LRI was assessed in several studies using a combination of blood culture, serology, and/or tracheal aspirate culture and ranged from <1%-25% (Table 2.2). Serology for pneumococcus has been found to be sensitive for the detection of bacteremic pneumococcal pneumonia in children, but these methods are insufficiently

validated for the detection of non-bacteremic pneumococcal pneumonia owing to the lack of a gold-standard comparator [225]. Pneumococcal pneumonia was diagnosed by serology in 8.3% of inpatient RSV-LRI cases with radiographic pneumonia [226], and in approximately one quarter of inpatient RSV-LRI cases with or without radiographic pneumonia [227, 228]. Tracheal aspirates, while not meeting the same specificity for etiology as blood cultures, may have higher specificity for pneumococcal disease than nasopharyngeal aspirates, and studies using these specimens reported a prevalence of pneumococcal pneumonia as high as 16% [218-221]. Pneumococcus was cultured from the expectorated sputum in a similar proportion (12.8%) of hospitalized RSV-LRI cases [229]. Interpretation of these results is complicated by the fact that both tracheal and sputum specimens can be contaminated with flora from the upper respiratory tract, and that findings from tracheal aspirates may not be representative of children who are not intubated.

We found only two studies that assessed prevalence of RSV among children with pneumococcal disease, both with significant limitations. One was a retrospective review of the hospital records of 82 children between the ages of 2 months and 18 years who were admitted to the hospital with IPD and were tested for viruses at the discretion of the clinician, with RSV detected in 5 (6%) [230]. The criteria for viral testing in these children were unknown and results were not stratified by age. The second study tested for RSV in children 7mo – 16 years with pneumococcal-associated radiologic pneumonia and found 10% positive, but most of these pneumococcal pneumonia cases were diagnosed by urine antigen testing only, which has low specificity for pneumococcal

disease in children given their frequent carriage of the bacteria, and age-stratified results were also not presented for this study [231].

Table 2.2 Co-occurrence of pneumococcal pneumonia in children with RSV-LRI

	Author, Setting, study type	Inclusion criteria	Specimen/test for bacterial detection	N	Serious bacterial infection n (%)[1]	Proportion of bacterial cases that were pneumococcus n (%)
Serology						
	Juven, low mortality, Finland [228] prospective	RSV-LRI[2] with fever or radiographic pneumonia	Serology	73	32 (44%); 18 (25%) Sp+[3]	18/32 (56%)
	Claesson, low mortality, Sweden [226] prospective	1mo-15 years, RSV pos and radiographic community acquired pneumonia	Serology (Sp only)	102	Inpatient RSV: 4/48 (8.3%) Sp+; outpatient 3/54 (5.6%) Sp+	n/a
	Korppi, low mortality, Finland [227] prospective	<5 yrs, with inpatient RSV- LRI	Serology	90	35/90 (39%); 20/90 (22%) Sp+	20/35 (57%)
Lower respiratory secretions						
	Resch, low mortality, Austria [222] prospective	<12 mo hospitalized with RSV-LRI	Blood, urine, stool, or tracheal aspirate culture or smears	464	17/464 (3.7%); 4/464 (0.9%) Sp+	4/17 (24%)
	Leung, low mortality, Hong Kong [232] (retrospective)	<18 yrs w RSV-LRI and ICU[4] admission	Blood and tracheal aspirate culture	117	14/117 (12%) 2/117 Sp+ (1.7%)	2/14 (14%)
	Hall, Low mortality, US [207] (prospective)	<3 yrs, hospitalized with RSV-LRI, excluding underlying conditions	Blood, tracheal aspirate, or cerebrospinal fluid culture, or urine rapid antigen test	565	7/565 (1.2%); 3/565 (0.5%) Sp+	4/7 (57%) (1 bacteremia, 3 pneumonia diagnosed by urine antigen)
	Thorburn, Low mortality, UK [218]	Children <5 yrs admitted to ICU with RSV-infection and lower respiratory secretions collected, nosocomial RSV infection excluded	ETT[5] culture (n=165 w specimen collected)	165	70/165 any growth (42%); 36/165 high growth ($>10^5$cfu /ml); 12/165 (7.3%) Sp+; 6/165 (3.6%) were high growth, $>10^5$ CFU/ml) Sp+	12/70 (17%) any growth; 6/36 (17%) high growth
	Duttweiler, Low mortality, Switzerland [219]	Infants admitted to ICU with RSV LRI	Tracheal aspirate culture	127	25/57 (44%); 9/57 Sp+ (16%)	9/25 (36%)

	Author, Setting, study type	Inclusion criteria	Specimen/test for bacterial detection	N	Serious bacterial infection n (%)[1]	Proportion of bacterial cases that were pneumococcus n (%)
	Kneyber, Low mortality, The Netherlands (retrospective) [220]	<12 mo inpatient RSV-LRI, admitted to ICU for respiratory failure or apnea	ETT culture	24	9/24 (33%) 0 (0.0%) Sp+	0/24 (0.0%)
	Randolph, low mortality, US (retrospective) [221]	Otherwise healthy children <36 mo admitted to ICU with confirmed RSV infection; excluded underlying conditions and preterm	ETT culture	47	13/47 (28%) samples showed probable bacterial pneumonia; 3/47 (6.4%) were Sp+	3/13 (23%)
Expectorated sputum						
	Hishiki, low mortality, Japan [229]	Children <5 with inpatient RSV-LRI	Expectorated washed sputum	188	82/188 (44%); 24/188 (13%) Sp+	24/82 (29%)

[1]Percentages rounded to first decimal place if <10%
[2] RSV lower respiratory illness
[3]Pneumococcus test positive
[4]Intensive Care Unit
[5]Endotracheal tube aspirate

A common feature of many of these studies of pneumococcal bacteremia and pneumonia in patients with RSV LRI is a reliance on retrospective review of hospital records, which presents the possibility of an initial viral diagnosis that has been changed to a diagnosis of bacterial infection once the latter was identified [209]. Another important limitation is their cross-sectional nature, which prevents them from being used to draw inferences regarding temporal associations. Furthermore, the majority of the investigations took place in low mortality settings, and as evidenced by the findings in Pakistan, may not be representative of higher mortality settings, where bacterial disease is more prevalent.

Very few studies have been designed to compare rates of pneumococcal disease in children with RSV-LRI to those without RSV-LRI. The few that have attempted to do so report mixed findings and have several limitations. One study of infants <2 months with fever (not necessarily associated with LRI) presenting to emergency departments found a lower incidence of bacteremia in infants with RSV-infection compared to those without RSV, but did not restrict the study population to children with LRI, and only 38% of infants with documented RSV infection had clinical bronchiolitis [209]. A study of bacterial and viral co-detection in children with radiographic pneumonia aged 6 months – 15 years found no association between the detection of RSV and pneumococcus in sputum samples, but had a very low overall detection rate for RSV (perhaps influenced by the older ages included) [233]. A study of inpatients with pneumococcal pneumonia and/or RSV-LRI with or without radiographic evidence of pneumonia, assessed for both pathogens using serology, and found increased pneumococcal pneumonia in children with RSV-LRI compared to those with non RSV-LRI [227], while another serological study of hospitalized children with pneumonia found no specific association between RSV-LRI and detection of pneumococcus [234].

Ecological studies show a temporal association between RSV-LRI hospitalizations and subsequent pneumococcal disease hospitalizations

Ecological studies have used hospitalization surveillance data to investigate a temporal relationship between peaks in RSV-LRI activity and cases of pneumococcal disease (Table 2.3). Several found a contemporaneous correlation between RSV-LRI and

pneumococcal hospitalizations [89, 235-238], and two found increased pneumococcal disease persisting for up to four weeks following peaks in RSV activity [89, 235]. Others found a 1.5 [90] or 4-week [239] lag between peaks in RSV activity and subsequent increases in incidence of pneumococcal disease. These lags are consistent with the hypothesis that RSV infection predisposes for increased risk of pneumococcal disease by altering the host respiratory environment. Only one study found no temporal association between RSV-LRI and pneumococcal disease in children [240], and one found a trend of IPD cases peaking slightly before RSV-LRI cases [241]. The only study to assess pneumococcal pneumonia in young children (rather than IPD) found a greater association between RSV hospitalizations and pneumococcal pneumonia compared to RSV hospitalizations and pneumococcal bacteremia [90]. The effect of age was not consistent across studies, with some finding stronger temporal associations in younger ages [90, 237, 238] but most observing a greater correlation between RSV hospitalization and pneumococcal hospitalizations in teens and adults compared to young children [235, 236, 239, 241].

Table 2.3 Temporal associations between RSV LRI and pneumococcal disease in ecological studies

Study, Location	Ages included	Years of study	Association	Adjustment for seasonality
Jansen, The Netherlands [241]	All	1997-2003	RSV and IPD positively associated in all age groups, IPD appeared to peak before RSV; after adjustment for season association was weakened in <18 yrs but still significant in adults	Yes, incidence of IPD during RSV season was compared to different seasonal baselines
Weinberger, United States [90]	Children <2	1992-2008	IPD cases peaked 1.5 weeks after RSV-LRI hospitalizations	Yes, harmonic term in regression analysis
Talbot, United States [235]	All	1995-2002	RSV associated with IPD in all ages within 4 weeks from the start of RSV peak activity; less pronounced in <18 yrs olds	No
Kim, United States [239]	All	1990-1993	IPD incidence peaked in infants and children 4 weeks after RSV peak, stronger association in adults	No
Ampofo, United States [89]	<18 yrs	2001-2007	Correlation between IPD and RSV up to 4 weeks after RSV peak activity	No
Murdoch, New Zealand [236]	All	1995-2006	RSV associated with increased IPD in children <5 years; overall 3-4% of IPD cases attributable to RSV across all ages	Yes, meteorological variables included in models
Nicoli, United Kingdom [240]	All	1996-2009	4% of IPD cases (all ages could be attributed to o RSV)	Yes, meteorological variables included in models
Weinberger, United States (Native American tribal lands) [237]	<7 yrs	1996-2012	15.5% bacteremic pneumonia attributable to bronchiolitis (RSV proxy); invasive non pneumonia infections not associated	Yes, sine and cosine terms to represent background seasonal factors
Watson, Australia [238]	All	2000	RSV activity correlated with IPD in children (no lag period), but not adults	No

Discerning the independent effects of RSV epidemics on pneumococcal epidemics is challenging due the fact that they tend to have shared seasonal patterns in temperate climates. Some studies therefore include temperature or other seasonal variables in their

analyses as a way to account for factors such as shorter photoperiods and lower temperatures that could increase the risk of transmission of both pathogens due to increased crowding and increased host susceptibility. In studies that took this approach, the correlations between RSV and IPD activity remained significant [90, 237, 241].

It is noteworthy that all of these studies were conducted in temperate climates where RSV and pneumococcal disease epidemics have shared seasonality, and it will be important to replicate them in tropical and sub-tropical settings with different patterns of weather and disease. One of the temporal association studies in the United States included data from Florida, which has an earlier and less pronounced RSV peak than the rest of the country as well as a sub-tropical to tropical climate. RSV activity and pneumococcal disease remained associated in Florida, but the lag between the two epidemic peaks was much longer (approximately 10 weeks) compared to the 1.5-week lag observed in other states [90]. A trend of pneumococcal disease peaking slightly after RSV epidemics appears to be consistent in a pneumonia etiology study in the high mortality setting of Pakistan, although it was not formally studied in that context [216].

Individual-level studies are critical for confirming the temporal associations found between RSV-LRI and pneumococcal disease in these ecologic studies. We are aware of only one individual-level study so far that has looked at this association (also in a temperate climate), and showed an increased risk of IPD following RSV-LRI hospitalization in children <2 years [242]. No association was found for increased risk of RSV-LRI hospitalization following IPD in the same population [242].

Increased pneumococcal carriage density in the nasopharynx is associated with RSV-LRI

Pneumococcal colonization of the nasopharynx is a prerequisite for the development of pneumococcal disease [243], and evidence indicates that it is facilitated by RSV-infection**.** Increased pneumococcal colonization prevalence [244, 245] as well as density [245] are associated with RSV in children <5 with LRI, with the strongest associations found in those <24 months old. A marginally statistically significant association between RSV (type B only) and pneumococcal carriage prevalence was found in a mixed population of children and adults with acute respiratory symptoms, but the analysis was not stratified by age [184]. In studies restricted to children with radiographic pneumonia there was no difference in pneumococcal carriage prevalence by RSV status, but RSV infection was significantly associated with increased pneumococcal density among those colonized [246-250]. In children <5 years hospitalized with WHO-defined severe or very severe pneumonia, RSV-pneumonia was not associated with increased pneumococcal carriage density, but children with RSV-pneumonia were more likely to have pneumococcal carriage densities above a threshold associated with confirmed pneumococcal pneumonia than children without RSV-pneumonia [249]. Neither rates of pneumococcal carriage, nor density, have been found to be elevated at 4-6 weeks following hospital discharge in children previously ill with RSV-LRI compared to the acute phase of the illness [246].

One study assessed the role of pneumococcal serotype in RSV-associated pneumococcal carriage in children with inpatient radiographic pneumonia, finding RSV-positive samples to be more frequently colonization by non-invasive serotypes and RSV-negative samples to be more frequently colonized with invasive serotypes [251]. This supports the hypothesis that invasive pneumococcal serotypes may be virulent enough to cause disease on their own, while non-invasive serotypes may require preceding viral illness in order to cause disease.

RSV-pneumococcal co-detection and increased severity

Overall, the available evidence indicates an association between co-detection of pneumococcal carriage with RSV-LRI and increased disease severity (Table 2.4). Pneumococcal carriage in children <2 years presenting to the emergency room with RSV-LRI is associated with higher clinical severity scores compared to those presenting with RSV alone [245], and the requirement for hospitalization with RSV-LRI is greater for those with nasopharyngeal microbiome profiles enriched for *Streptococcus* species, independent of age [74]. In the same study, increased expression of genes associated with the inflammatory response were also found in the *Streptococcus* enriched microbiome profiles, indicating that microbial co-signaling may contribute to enhanced neutrophil recruitment and activation, which in turn leads to more severe illness [74]. In another microbiome study, however, *Streptococcus* dominant nasopharyngeal profiles were not associated with increased risk of fever in children <1 with RSV-LRI [165]. In hospitalized children <2 years with RSV-LRI, pneumococcal colonization was associated with a requirement for supplemental oxygen, and pneumococcal density was positively

correlated with RSV viral loads, but high pneumococcal colonization density associated with reduced requirement for mechanical ventilation [246]. Notably, children that required mechanical ventilation also tended to be younger and have less daycare attendance than the other two groups, which are factors associated with reduced pneumococcal carriage [246].

Table 2.4 Association between detection of pneumococcal carriage in the nasopharynx and RSV-LRI disease severity

Study	Study Pop	Spn[1] detection	RSV detection	Severity marker	Association
Brealey [245]	<2 yrs presenting to ER w LRI	NP[2] PCR	NP PCR	Clinical asthma score	Significantly higher clinical severity score in children with RSV+Spn than with one pathogen in the absence of the other
Teo [165]	<1 year birth cohort at high risk of atopy, samples collected at regular visits and at ARIs	16s RNA sequencing, and PCR Species level ID only	NP PCR	Fever with LRI	Streptococcus not associated with fever, among those with RSV-LRI
Vissers [246]	<2 yrs hospitalized with RSV-LRI	NP PCR	NP PCR	Mild illness = no hypoxia Moderate illness = received supplemental oxygen Severe illness = required mechanical ventilation	No difference by severity with pneumococcal colonization, but higher Spn density associated with LESS severe clinical disease
Wouter [74]	< 2 yrs with first episode of MALRI (outpatient or inpatient)	16s RNA sequencing, and PCR Species level ID only	NP PCR	Requirement for hospitalization	Nasopharyngeal microbiota of RSV-infected children enriched by *Streptococcus* spp. associated with increased severity compared to those with another microbiota composition.

[1]*Streptococcus pneumoniae*
[2]Nasopharyngeal

Co-detection of RSV and pneumococcus in the lung is rare

RSV and pneumococcus are rarely co-detected in lung aspirates (LA), even in settings of high pneumococcal disease burden. Lung aspirates are the gold standard specimen for determining LRI etiology, but are not commonly collected, and most data comes from older studies where viral testing was not conducted [252]. Many LA studies in children with pneumonia that incorporated viral testing by traditional culture techniques have

reported no viruses in the lung [253-257]. Others found only non-RSV viruses [258-260]. Studies using traditional culture methods and, more recently, PCR, have been conducted in the high pneumonia burden settings of the Gambia, Papua New Guinea, and Malawi. These investigations, conducted from 1984 through the present, have concluded that co-infection of the lung with pneumococcus and RSV does occur, but is a relatively rare occurrence, even in the context of high pneumococcal pneumonia disease prevalence [261].

In the Gambia, lung aspirates were collected from 94 children aged 3mo – 5yrs with radiographic pneumonia with clear consolidation that could be reached by a needle. Pneumococcal pneumonia was confirmed by blood, pleural or lung fluid culture in 15% of the study population. Less than half (46%, [43/94]) of LA specimens were positive for any virus or bacteria, with 4% (4/94) positive for RSV by culture. Two of the RSV-positive LA specimens were from a case with pneumococcal disease, indicating that 2/94 (2%) patients had probable co-infection of the two pathogens in the lung that was detectable by these traditional diagnostic methods [261]. In Papua New Guinea, 18/83 (22%) of children <12mo with radiographic pneumonia had pneumococcus isolated from blood or lung fluid. RSV was isolated in culture from 1/62 (2%) of lung aspirates but it was not reported whether or not pneumococcus was isolated from the same child [262].

RSV is more frequently detected in lung aspirate studies that incorporate PCR testing. In the Gambia, 90% (47/52) of children aged 2-59 months with radiographic pneumonia and clear consolidation had LA collected. Forty-eight (91%) of the children had

pneumococcus detected by molecular methods in lung or pleural aspirates (results not disaggregated by specimen type) and 19% (10/52) had a virus detected in the presence of bacteria. The frequency of specific bacterial-viral combinations was not specified, but RSV was noted as the most commonly detected virus in the lungs [263]. In 95 Malawian children aged 2mo-15 years (median age 2.6 years) with radiological consolidation, pneumococcus was detected in the blood and/or lungs of 39% of cases, but RSV was not detected in any cases [264].

Given the disproportionate burden of severe RSV-illness in young infants, comparisons across case series of different ages such as those reported here should be made with caution. It is also important to note that children who have lung aspirates collected represent a clinically distinct subset of children presenting with severe lower respiratory tract infection, and are not necessarily of the full etiologic spectrum of radiologic pneumonia.

Co-detection of RSV and pneumococcus in lung autopsy studies

The limited available data from lung autopsy studies in children provide no supporting evidence for an interaction between RSV and pneumococcus. Postmortem lung tissue investigations can increase the identification of causative pathogens in cases of fatal lower respiratory disease and can provide confirmation of antemortem laboratory diagnoses, but such data, especially from children, are extremely limited [265]. Autopsy studies of 264 Zambia children aged 1-16 years who died of respiratory disease identified

RSV by histology staining in two fatal cases; whether or not either of these cases was co-infected with pneumococcus was not reported [266].

Vaccine probe studies

Evidence for causal associations between RSV and pneumococcus in LRI can most efficiently be explored through probe studies using vaccines or monoclonal antibodies [186, 267]. One vaccine trial has directly assessed the impact of nine-valent pneumococcal conjugate vaccine (PCV-9) on the rate of RSV-pneumonia in South Africa [268, 269]. In the trial, a diagnosis of RSV-associated pneumonia required either clinical or radiological evidence of pneumonia plus a RSV-positive immunofluorescence assay result from nasopharyngeal aspirate [269]. PCV efficacy for prevention of RSV-associated pneumonia was 32% (95%CI: 6%, 50%) among HIV-uninfected children <3 yrs in a per protocol analysis of fully immunized participants (in the per-protocol analysis of all children, inclusive of those with HIV, the trend was similar, but not statistically significant, with 22% efficacy for prevention of RSV-associated pneumonia (95% CI: -3, 41) [268]. There was no demonstrated efficacy of PCV for all-cause or viral-associated bronchiolitis. This study provides quantitative evidence of the importance of pneumococcal co-infection in virus-associated pneumonias in hospitalized children and highlights the limitations of using blood cultures to identify this association. Of the 199 children with RSV-LRI in the control arm of the trial, only 3 (1.5%) had vaccine-type pneumococcal bacteremic pneumonia detected, despite the fact that 14 (7.0%) likely had vaccine-type pneumococcal disease based on vaccine attributable rate reduction for RSV-

LRI [268]. The association was observed despite a transient increase in RSV-LRI hospitalizations in the PCV arm for one to eight days after vaccination, which the investigators speculated may have be due to increased susceptibility to pneumococcal pneumonia among those already colonized with pneumococcus and infected with RSV, potentially due to a vaccine-induced depletion in pneumococcal capsular-antibody-specific B cells before the development of opsonophagoctyic antibodies [269].

In a randomized trial of 13-valent pneumococcal conjugate vaccine (PCV-13) in the Gambia, bronchiolitis (without RSV confirmation, defined as clinical pneumonia with wheeze detectable on auscultation without dullness to percussion, bronchial breathing, or radiographic pneumonia) was reduced by 39% in 2-11 month olds and 19% in 12-24 months following vaccination [270]. Although children with bronchiolitis in the Gambia were not tested for RSV, this is consistent with the magnitude of the RSV-specific reduction observed in the South Africa PCV trial.

A time series analysis of IPD and RSV hospitalizations reported an 18% reduction in RSV-coded hospital admissions in children <3-11 mo in the US following PCV-7 introduction (but no decline in children <3 months) [90]. It is possible that pneumococcal disease was driving these hospitalizations, a proportion of which were RSV-coinfected, with fewer cases of RSV being detected as a result of the decreased IPD incidence [90]. Consistent with this, a trend in declining in RSV-LRI hospitalization following PCV introduction in Alaska was also observed [271]. In western Australia, a review of hospital records found a 16% (95%CI 6-24%) reduction in RSV hospitalization rates in aboriginal

children and a 6% (95% CI 1-11%) reduction in RSV hospitalization rates in non-aboriginal children <5 years of age, following PCV introduction (calculated from [272]).

There is not yet a licensed vaccine for RSV, which would allow for an investigation of the impact of RSV prevention on rates of subsequent pneumococcal disease, but future studies could be designed to assess this relationship. An evaluation of a completed RSV immunoprophylaxis randomized trial [140] is currently under way for this purpose, with pneumococcal density in the nasopharynx being used as a proxy for risk of pneumococcal disease.

Summary and future directions

A convincing and growing body of evidence suggests that RSV and pneumococcus interact synergistically in children with lower respiratory illness. While much of the evidence supports the hypothesis that preceding RSV infection contributes to subsequent pneumococcal disease, there are also signals of a potential a bi-directional relationship between the two pathogens that merits further investigation. It is apparent from this review that more individual-level data in carefully defined study populations are needed to better understand the implications of the interaction between RSV and pneumococcus on LRI in children. Additionally, there is some evidence that RSV-pneumococcal interactions may be mediated in part by serotype, though this requires further investigation both in experimental and epidemiological studies.

Mechanisms for interactions between RSV and pneumococcus in the respiratory epithelium are well described by experimental studies, and animal models show increased disease severity with RSV and pneumococcal co-infection compared to infection by pneumococcus alone. More research is needed, however, to understand the implications of sequence, timing and dosage of exposure to these two pathogens.

Among observational studies, the strongest evidence for an association between RSV-LRI and pneumococcal disease comes from (1) studies showing a temporal correlation between RSV-LRI hospitalizations and IPD hospitalizations, including an individual-level study of children in Sweden, and (2) investigations of children with severe RSV-LRI that use pneumococcal carriage density as a proxy indicator for risk of pneumococcal pneumonia. The majority of pneumonia etiology studies assessing co-infection with RSV and pneumococcus are retrospective, cross-sectional, case series studies that offer little insight into an association between the two pathogens. The cross sectional nature of most etiology studies is a weak design for the evaluation of the causative association between RSV and pneumococcus, particularly given that RSV virus preceding bacterial infection may have cleared by the time the child develops illness severe enough to warrant medical attention. Attempts to gain insights from etiologic studies of children with pneumonia are further challenged by reliance on upper respiratory tract specimens that are not specific for bacterial infection in the lung, and the low sensitivity of blood culture for pneumococcal pneumonia. Were they more readily available, lung aspirate and autopsy studies using advanced diagnostics might shed more

light on the frequency of co-infection of the lung with these two pathogens versus either pathogen alone.

A gold-standard study to investigate interactions between pneumococcus and RSV could be a randomized trial of an RSV-immunoprophylaxis or vaccine candidate that includes respiratory tract sampling periodically starting from birth with regular sampling of the upper respiratory tract for RSV and pneumococcal carriage, continuing through early childhood, and with standardized collection of specimens and clinical data at all respiratory illness events.

In the absence of such a potentially resource-intensive investigation, conventional vaccine probe studies offer potential for assessing causal associations between RSV and pneumococcus, and have several advantages over observational studies. Evidence from pneumococcal conjugate vaccine probe studies provide a strong basis for the argument that future pneumococcal vaccine and RSV immunoprophylaxis / vaccine trials should include testing for RSV-LRI and pneumococcal disease, respectively, as part of their assessment of primary endpoints. This would contribute greatly to our understanding the broader impact of these interventions and to making the investment case for RSV vaccines and sustained investment case for pneumococcal vaccines.

With the exception of the PCV trials, a consistent feature of many of the epidemiological studies of associations between RSV-LRI and pneumococcal disease is that they have taken place in predominantly low-mortality, high-income settings. Additional data are

needed from settings where the population is at greater risk of disease from both pathogens and where underlying host risk factors, co-morbidities, and environmental risk factors may contribute to increased rates of co-transmission and to the clinical significance of co-infection of the lungs with RSV and pneumococcus.

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Chapter 3: Thesis Objectives

Objective 1: To evaluate the role of RSV MALRI prevention on the prevalence and density of *Streptococcus pneumoniae* carriage in the infant nasopharynx

Background:

Respiratory syncytial virus (RSV) and *Streptococcus pneumoniae* are common causes of lower respiratory illness (LRI) in children. A growing body of evidence suggests that these two pathogens may act synergistically in the development of LRI, with greater incidence and severity of disease occurring with both pathogens than with either alone. Recently, a phase three double blinded randomized trial of a next-generation RSV monoclonal antibody, motavizumab, showed high efficacy for the prevention of RSV associated medically attended lower respiratory illness (MALRI) in a population of healthy full term infants. We used samples collected at these MALRI events to assess whether *S. pneumoniae* nasopharyngeal carriage, a necessary precursor to the development of pneumococcal LRI, was associated with RSV MALRI and if motavizumab prophylaxis altered its prevalence and density.

Methods:

Infants less than six months of age by December 31st of any of the four years of enrollment in the motavizumab trial were enrolled and prophylaxed with monthly doses of motavizumab or placebo for five total doses through the RSV season (150 days following randomization). Nasopharyngeal secretions (washes or aspirates) were collected at every MALRI for the first 150 days following randomization and tested for

RSV. We tested stored samples from these events for *S. pneumoniae* carriage by quantitative PCR.

Results:

We found no difference in prevalence of S. *pneumoniae* carriage by RSV status (65.6% of RSV associated MALRI and 64.9% of non-RSV MALRI had *S. pneumoniae* detected, (p=0.87)), but mean carriage density was greater in RSV associated MALRIs compared to those without RSV(6.01 log(10) copies/mL vs. 5.73 log(10) copies/mL, p=0.03). The proportion of events with pneumococcal carriage density greater than 6.9 log(10) copies/mL was also greater in RSV-associated MALRI compared to non RSV MALRI (14.8% vs. 9.0%, p=0.03).There was a corresponding reduction in the density of pneumococcal carriage at MALRI events that occurred in the motavizumab treatment group compared to the placebo treatment group, although this did not reach statistical significance.

Discussion:

Our results support the hypothesis that *S. pneumoniae* and RSV may interact synergistically and suggest that preventing RSV illness with a monoclonal antibody, or potentially a vaccine, in infancy may decrease rates of high-density pneumococcal carriage in the nasopharynx. This may in turn reduce pneumococcal transmission in communities, as well as lowering the risk of development of pneumococcal disease. Future studies of RSV immunoprophylaxis products and vaccines should consider measuring rates of pneumococcal disease as outcome measures to evaluate this association.

Conclusion:

Preventing RSV lower respiratory illness in infancy likely reduces pneumococcal carriage density in the infant nasopharynx, and this may lead to a corresponding reduction in pneumococcal transmission and disease.

Objective 2: To evaluate the impact of RSV MALRI prevention in infancy on MALRI with other respiratory viruses, and on subsequent medically attended wheezing at ages one to three years

Background: A double-blinded placebo controlled randomized trial of motavizumab in healthy, full term infants reported high efficacy against respiratory syncytial virus (RSV) inpatient and outpatient medically attended lower respiratory illness (MALRI), but no efficacy against subsequent medically attended wheezing through 3 years of age. We evaluated the risk of non-RSV MALRI and the basis for the lack of efficacy against subsequent wheezing.

Methods: Infants less than six months of age by December 31st of any of the four years of enrollment in the motavizumab trial were enrolled and prophylaxed with monthly doses of motavizumab or placebo for five total doses through the RSV season (150 days following randomization). We tested stored nasopharyngeal specimens from MALRIs occurring during the first 150 days of the trial follow up period by viral multiplex and *Streptococcus pneumoniae* PCR. Human rhinovirus (HRV) positive samples were subtyped. We evaluated these and other exposures for medically attended wheeze at ages 1-3 years of age (subsequent wheeze).

Results: Motavizumab reduced MALRI with RSV alone and in the presence of other viruses in inpatients [RR 0.13 (95%CI: 0.06, 0.24) and RR 0.12 (95%CI 0.05, 0.25), respectively], and in outpatients [RR 0.15 (95%CI 0.07, 0.30) and RR 0.48 95%CI (0.30, 0.79), respectively]. Rates of outpatient influenza type A (Flu A) and human metapneumovirus (HMPV) associated MALRIs were increased in motavizumab compared to placebo participants. A family history of asthma, exposure to children in

daycare, and MALRI with HRV subtype A and C, parainfluenza virus (PIV), or coronavirus during the first 150 days of follow up, were independently associated with subsequent wheeze at ages 1-3 years. RSV (inpatient) MALRI was associated with subsequent wheeze in the motavizumab group (i.e. children who broke through motavizumab prophylaxis) but not the placebo group.

Discussion: Motavizumab prevents MALRI with RSV alone and in combination with other viruses. The comparatively reduced efficacy for outpatient RSV MALRI with other viruses suggests that that as disease severity is reduced, RSV test-positivity becomes less specific for LRI causality, which has implications for future efficacy trials. RSV MALRI was not independently associated with subsequent medically attended wheeze at ages 1-3 years in this study population, but PIV, coronavirus and HRV MALRIs were. Participants who experienced motavizumab break through were at significantly increased risk of subsequent wheeze after adjusting for other risk factors, and may represent a subgroup of children at high risk both for severe disease given RSV infection and for subsequent wheeze, regardless of RSV-illness exposure.

Conclusion: Globally, RSV is associated with significant child morbidity, particularly in early infancy, but the role that these severe illness episodes play in the causal pathway to wheeze and asthma in later childhood is unclear. We found that motavizumab prevents MALRI with RSV alone as well as MALRI with RSV in combination with other viruses, and that certain non-RSV viral MALRIs in infancy, particularly rhinovirus, PIV and coronaviruses, may increase the risk of subsequent wheezing in some settings. Additionally, there may be a subgroup of children at high risk for both

immunoprophylaxis failure and risk of wheezing in early childhood, and this merits further investigation.

Objective 3: To evaluate the risk of RSV MALRI in the second year of life following the prevention of RSV MALRI with motavizumab immunoprophylaxis in infancy

Background:

Respiratory syncytial virus (RSV) associated lower respiratory illness is a leading cause of child morbidity and is most severe in early infancy. To what extent this is attributable to young age and to what extent it is due to the experience of a primary infection is not fully understood. Recently, a phase three randomized trial of a next-generation RSV monoclonal antibody, motavizumab, was shown to have high efficacy for the prevention of inpatient and outpatient RSV-associated medically attended lower respiratory illness (MALRI) in a population of healthy full term infants during their first winter RSV season. We assessed whether there was increased risk of RSV MALRI in this population in the second RSV season following RSV MALRI prevention in the first season.

Methods:

Infants less than six months of age by December 31st of any of the four years of enrollment in the motavizumab trial were enrolled and prophylaxed with monthly doses of motavizumab or placebo for five total doses through the first winter RSV season (150 days following randomization). Nasopharyngeal samples were collected at every MALRI for three years following enrollment in the motavizumab trial. We tested stored nasopharyngeal samples collected at MALRI events that occurred during the RSV season of the second year of life for RSV A and B using real-time PCR.

Results:

We observed no increased relative risk of RSV-MALRI events in the second season for the motavizumab group compared to the placebo group (RR 1.09 (95%CI 0.79, 1.50, equivalent to a <1% increase in absolute risk). Participants with RSV MALRI in the first RSV season were less likely to have an RSV MALRI, but more likely to have a non-RSV MALRI in the second RSV season compared to participants with no RSV MALRI in the first season.

Discussion:

We found no statistical difference in rates of medically attended RSV illness, either inpatient or outpatient or both, by treatment group in the second RSV season. This reassures that there is not a substantial increased risk of medially attended respiratory events attributable to RSV in the second year of life among children who had protection against RSV disease as infants. We did observe a 9% relative increase in the rate of any RSV MALRI in the second RSV season for the motavizumab treatment group compared to the placebo treatment group that was not statistically significant, and which corresponded to a <1% absolute increase in the rate of any RSV MALRI. We also observed a trend of decreased severity of RSV MALRI in the second season compared to the first season for both treatment groups. The proportion of MALRI events in the second RSV season with samples collected within the analytic window that were available for RSV testing reduced our statistical power to detect true differences in rates of RSV MALRI between treatment groups in this time period. However, the small magnitude of the increase in risk that we observed in the motavizumab group, combined with less

severe RSV MALRI in the second compared to the first year of life, provides strong support for the benefit of delaying primary RSV lower respiratory illness beyond infancy.

Conclusion:

Young age and the experience of a primary infection are both thought to contribute to elevated risk of severe illness with RSV in infancy. We found no significant increase in RSV MALRI in the second RSV season after preventing RSV MALRI with motavizumab in the first RSV season. Our results support the argument of a significant overall public health benefit to delaying the primary lower respiratory illness until the second year of life.

Chapter 4: Methods

This thesis research rests on the foundation of a phase 3 randomized double-blind placebo controlled clinical trial of the RSV monoclonal antibody motavizumab that was conducted by the Center for American Indian Health (CAIH) at the Johns Hopkins Bloomberg School of Public Health in partnership with MedImmune, the study sponsor. Methods for the clinical trial are described in section 4.1 below; methods for the additional data collection that makes up this thesis work are described in section 4.2.

4.1 Methods for the motavizumab clinical trial

From 2004 - 2009, CAIH conducted a phase-3 efficacy trial of the motavizumab RSV F monoclonal antibody under the direction of principal investigator Kate O'Brien. The trial was sponsored by the manufacturer (MedImmune) and was designed to evaluate the safety and efficacy of motavizumab for the prevention of medically attended RSV-associated respiratory disease, otitis media, and subsequent wheezing in a population of healthy, full-term American Indian infants. This trial is the only study to date that has evaluated RSV immunoprophylaxis in a population of healthy full-term infants. All other trials have been conducted in preterm infants or those with medical conditions putting them at high risk of RSV disease.

Study Population

Study participants were infants from three southwest American Indian tribes: the Navajo, the White Mountain Apache, and the San Carlos Apache. RSV hospitalization rates

among infants and young children in these communities have been shown to be nearly 2-5 times higher than among the general U.S. population, and are similar to those of infants whose medical condition puts them at high-risk for RSV disease. These latter infants are now eligible for palivizumab prophylaxis according to policies of the American Academy of Pediatrics and the Advisory Committee on Immunization Practices (Table 4.1.1) [110]. Participants were enrolled from 11 research sites within the three participating Indian reservations (Figure 4.1.1).

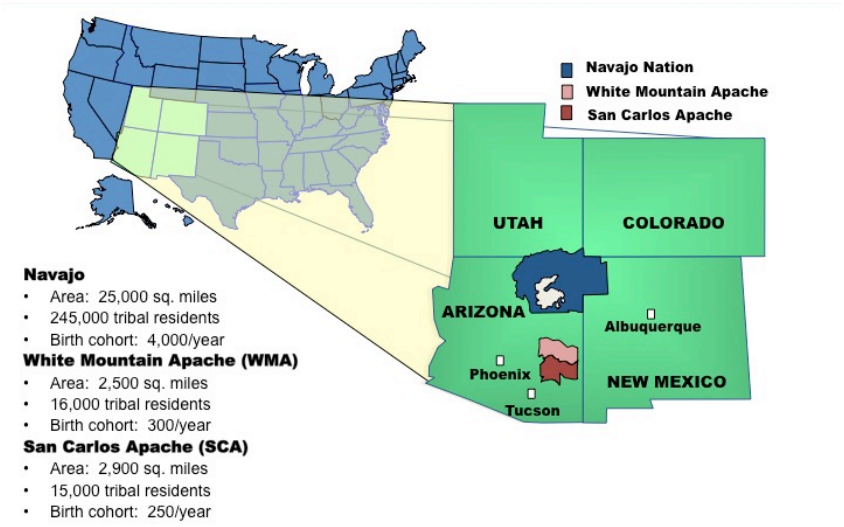
Table 4.1.1 American Academy of Pediatrics (AAP)[1] red book committee recommendations for Palivizumab (2014)[2] [123]

High Risk Population	AAP Recommendation
Premature infants (<29 weeks gestation) who are younger than 12 months at the start of RSV season	Palivizumab prophylaxis during the RSV season in the first year of life (a maximum of 5 monthly doses of 15 mg/kg; qualifying infants born during the RSV season may require fewer doses)
Infants born at <32 weeks gestation with chronic lung disease of prematurity requiring ≥28 days of supplemental oxygen after birth, or certain chronic heart diseases, or airway clearance issues	Same as above
Infants born at <32 weeks gestation with chronic lung of prematurity requiring ≥28 days of supplemental oxygen after birth and who continue to require medical intervention during the 6-month period prior to the second RSV season	Palivizumab prophylaxis during the RSV season in the first and second years of life
Children younger than 24 months who will be profoundly immunocompromised during the RSV season	Palivizumab prophylaxis considered during the RSV season

[1]The Advisory Committee on Immunization Practices refers to these AAP guidelines in their recommendations

[2]Due to the high burden of RSV disease and costs associated with transport from remote locations, a broader use of palivizumab may result in Alaska Native and possibly other selected American Indian populations.

Figure 4.1.1 Navajo Nation, White Mountain Apache and San Carlos Apache Indian Reservations



Study Design

The study was a phase three, double-blind, placebo-controlled, 2:1 randomized trial. Participants randomized to the intervention arm received five intramuscular doses of motavizumab (15 mg/kg), 30 days apart. Participants randomized to the control arm received a visually identical placebo dosed on the same schedule. The primary aim of the trial was to assess whether motavizumab could safely and effectively reduce the incidence of serious RSV disease requiring hospitalization in the Native American full term infant population. It also assessed whether motavizumab could prevent outpatient RSV medically attended acute lower respiratory illness (MALRI), medically attended otitis media (OM) and medically attended wheezing through 3 years of age. The originally planned study sample size was 2,100-3,000 participants, providing statistical power between 77% and 90% (2-sided alpha 0.05) to observe a 45% reduction in RSV

hospitalization, assuming a true RSV hospitalization rate of 6% in the placebo group and 3.3% in the motavizumab group.

Study Drug

Motavizumab (MEDI-524) was shown to have enhanced potency over palivizumab and to have the potential for an anti-RSV effect in both the upper and lower respiratory tract in preclinical trials. All participants were evaluated monthly for the first five months (150 days following randomization) for adverse events and for serum collection and evaluations.

Participants

The period of enrollment was October – December, 2004-2007 (four calendar-year RSV seasons included in the study, in total). Participants were healthy infants of ≥36 weeks gestational age who were less than six months of age by December 31st of any of the four years of enrollment. Exclusion criteria in addition to <36 weeks gestational age included chronic lung disease of prematurity, chronic heart disease, long-term hospitalization, current or past wheezing, current RSV infection, receipt of palivizumab or any other antibody within the three months prior to randomization, as well as other serious medical conditions specified in the study protocol.

Clinical Endpoints and Study Follow up

Participants were followed for 150 days following the first dose of motavizumab or placebo (study day 0) for medically-attended respiratory events, with RSV-associated

hospitalizations as the primary study endpoint (defined below). Nasopharyngeal secretions were collected at every MALRI event. All specimens collected during the 150-day follow up period were tested for RSV by RT-PCR. Following study day 150, participants continued to be followed for medically-attended respiratory events through age three years, with nasopharyngeal specimens collected and stored but not tested until now for any respiratory pathogens.

Specimen collection and testing

The collection of nasal wash secretions involved instilling 15 – 20 cc of Ringer's lactate solution into each nostril of a seated child with a bulb syringe and collecting it from the opposite nostril. In children who could not have a nasal wash specimen collected, a nasal aspirate was obtained by instilling 3 – 6 cc of sterile saline into the nose and withdrawing nasal mucus using a feeding tube with a suction device. One milliliter of nasopharyngeal specimen was mixed with 6 ml viral transport medium and then divided into 4 – 8 aliquots which were snap frozen immediately using liquid nitrogen or an ethanol/dry ice bath, and stored at -70°C. At facilities where snap freezing was not possible, aliquots were immediately stored at -80°C. After freezing, aliquots were shipped to central laboratories for storage. Those collected within 150 days of randomization (the RSV season) were tested for RSV A and B by PCR assay. Aliquots of untested specimen remained in storage at -80°C with continuous temperature monitoring. Nasopharyngeal secretions collected within +/- five days of the MALRI event date (hospital admission for inpatient events; doctor visit date for outpatient events) were considered to be within the analytic window and were included in the analysis. Whenever possible, study staff

attempted to collect the specimens within +/- three days of the event. In instances where nasopharyngeal secretions were not collected in the facility, a study nurse would attempt collection at a home visit.

Clinical Endpoints and Case definitions:

Primary study endpoint for the trial:

- RSV –associated hospitalization and/or death (study days 0 – 150)

Secondary study endpoints for the trial:

- RSV outpatient MALRI episodes (study days 0 – 150)
- Otitis media (physician diagnosed acute OM, acute tympanic membrane perforation, bulging tympanic membrane, red tympanic membrane with fever, OM with effusion, or acute middle ear effusion)
- Subsequent medically attended wheezing events with discharge diagnosis of asthma, bronchiolitis, reactive airways disease, or if wheezing was documented by examining physician (from age 1-3 years)

<u>RSV hospitalization (inpatient RSV MALRI) case definition:</u>

An RSV hospitalization was defined as either 1) a respiratory hospitalization with a positive RSV PCR test within the analytic window (primary hospitalization) or 2) new onset of lower respiratory symptoms in an already hospitalized child, with an objective measure of worsening respiratory status, such as new requirement for supplemental hospitalization, increase in supplemental oxygen requirement from prior to the onset of

lower respiratory illness symptoms, or need for new mechanical ventilation, along with a positive RSV PCR test (nosocomial RSV inpatient event).

Outpatient RSV MALRI case definition:

Outpatient lower respiratory tract illness events were reviewed for inclusion by study investigators and were defined as a medical diagnosis of bronchiolitis or pneumonia. In the absence of such a medical diagnosis, the occurrence of the lower respiratory illness was determined by the study investigator's review of the medical records for the presence of lower respiratory signs and symptoms including cough, retractions, ronchi, wheezing, crackles or rales, as well as associated signs or symptoms including coryza, fever and apnea. As with RSV hospitalizations, outpatient RSV illness was confirmed by PCR testing.

Definition of wheeze:

Participants were followed from study day 0 (day of first study drug) through three years of age for the occurrence of medically attended wheezing events. Medically attended wheezing was counted as an outcome event if there was a discharge diagnosis of asthma, bronchiolitis, reactive airway disease, or documentation of wheezing in the medical record by the treating physician. A new wheezing episode was defined as one that occurred more than two weeks after the diagnosis of the previous episode and did not represent a persistence of the previous episode according to medical opinion. Only new wheezing episodes were included in the wheezing analyses.

Subsequent wheeze events were those medically attended wheeze events occurring between one and three years of age. Another approach would have been to count subsequent wheeze events as those medically attended wheeze events occurring after 150 days of follow up (i.e. following the RSV season). However, given that children could have been enrolled between birth and six months of age, they would have been different ages at the end of the RSV season. The one to three year age range was therefore selected so that all children would be assessed for subsequent medically attended wheeze outcomes within the same age interval, which was also a simpler outcome for the purposes of reporting and comparing to other studies.

Subsequent medically attended wheeze was classified using three outcome definitions: (1) ≥1 medically attended wheeze event, (2) serious early childhood wheeze, and (3) recurrent wheeze. Serious early childhood wheeze and recurrent wheeze are two overlapping but distinct subsets of medically attended wheeze. A child was considered to have serious early childhood wheeze if s/he met any one of four conditions between one and three years of age: (1) three or more attended wheezing events during any 12-month period, (2) a need for one or more courses of systemic steroids for treatment of a medically attended wheezing event, (3) a need for asthma control medications over a 12-month period for at least three consecutive months (i.e., ≥90 days) or five cumulative months (i.e. ≥150 days), with duration assessed by a combination of parental interviews and medical records, or (4) a least one inpatient wheezing event. Recurrent medically

attended wheeze was defined as three or more medically attended wheezing events during any 12-month period between one and three years of age.

Analyses

Intention-to-treat analyses were the primary analyses for the trial, with participants analyzed according to treatment group of randomization. According-to-protocol analyses were also conducted including participants who received ONLY motavizumab or placebo (either prior to meeting primary endpoint definition or for all five doses). Participants who were not followed through day 150 (lost to follow up) and did not have a hospitalization event prior to this were counted as not having met the RSV hospitalization endpoint; Kaplan-Meier analyses were done to account for the reduced length of follow-up for some participants.

Study Results

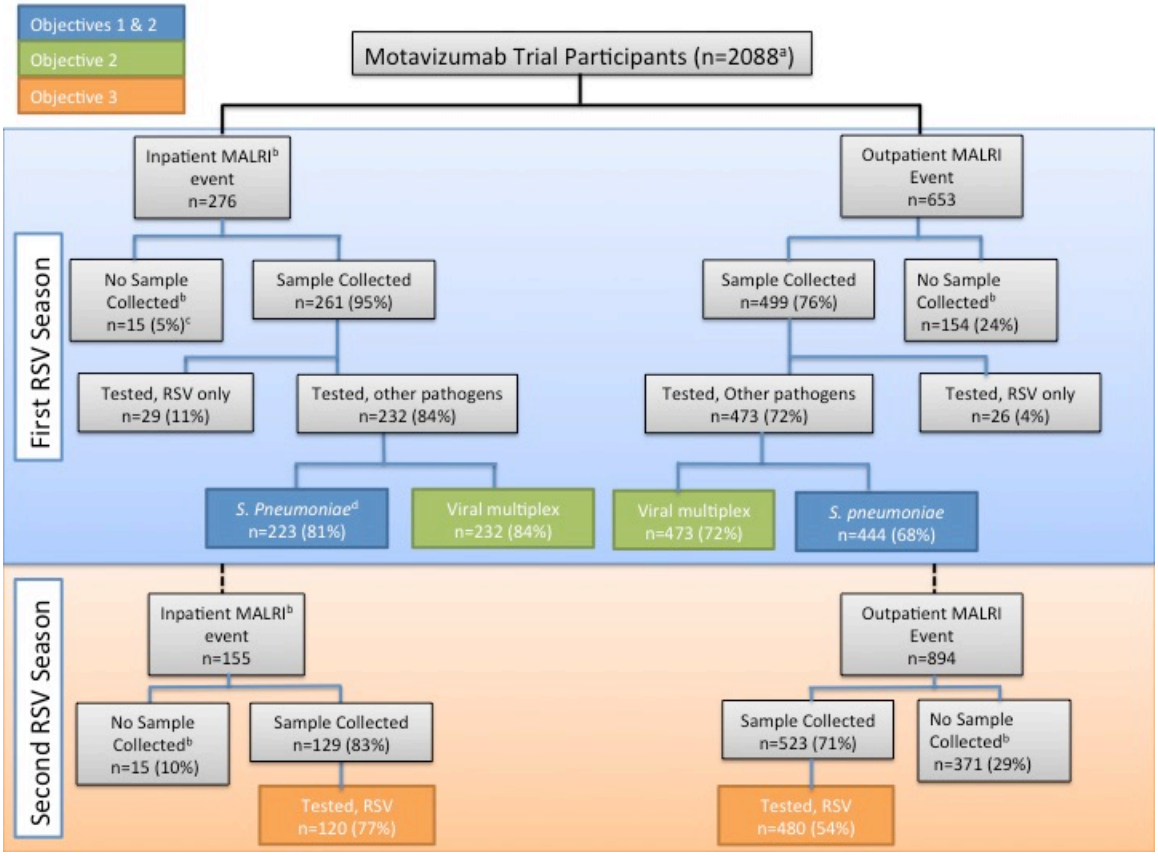
A total of 1,417 infants were randomized to the motavizumab intervention group and 710 were randomized to the control group (2,127 participants total). Among the motavizumab participants, 21/1,417 (1%) experienced an RSV hospitalization, compared to 80/710 (11%) in the placebo group (relative risk (RR) 0.13 (95% 0.08, 0.21)). There was an overall 50% rate reduction in all-cause hospitalization for respiratory illnesses. Outpatient RSV-MALRI occurred in 41/1,417 (3%) of motavizumab participants compared to 71/710 (10%) of placebo participants (RR 0.29 (95% CI 0.20-0.42)). The proportion of non-RSV MALRI inpatient and outpatient events was slightly higher in the motavizumab group compared to the placebo group, but the difference did not reach statistical

significance. Overall, motavizumab reduced the proportion of patients with any medically attended RSV ALRI by 80% (RR 0.20, (95% CI 0.15, 0.27)) from 21.3% (151/710) to 4.3% (61/1,417). Importantly, there was no difference by treatment group in medically attended wheeze events between the ages of 1 and 3 years. Results from the according-to-protocol analyses were consistent with the intention-to-treat analyses.

4.2 Methods for this thesis research

This thesis research used stored, untested, nasopharyngeal specimens collected at inpatient and outpatient MALRI events that occurred during the motavizumab clinical trial (the parent study). Informed consent for the parent study was obtained from a parent or guardian of participants. The Navajo Nation IRB, the Phoenix Area Indian Health Service IRB, the San Carlos Apache IRB, and the Johns Hopkins Bloomberg School of Public Health IRB provided ethical approval for the parent study. The original consent documents included permission to test the nasopharyngeal specimens collected at medically attended respiratory illnesses for other respiratory pathogens in addition to RSV. We obtained additional approvals from the Navajo Nation IRB and the Indian Health Services IRB for the testing of non-RSV respiratory pathogens that took place at the University of Wisconsin-Madison and the Centers for Disease Control and Prevention Arctic Investigations Program laboratory. Approval for additional testing of specimens from San Carlos Apache participants was not sought, and their specimens were excluded from testing. We therefore included 2,088 of 2,127 of the original trial participants in the analyses presented here (Figure 4.2.1).

Figure 4.2.1 Specimen testing subsequent to motavizumab clinical trial primary analysis



[a] Medically attended acute lower respiratory illness
[b] No sample collected within the analytic window
[c] All calculated proportions are taken using the total number of events above as the denominator

Objective 1 Methods

The first research objective was to assess the relationship between RSV MALRI prevention and pneumococcal carriage and density. For this objective, stored nasopharyngeal specimens collected at MALRI events in the first RSV season were tested for *Streptococcus pneumoniae* by a quantitative real-time PCR assay. The testing was done at the Centers for Disease Prevention and Control, Division of Preparedness and Emerging Investigations, Arctic Investigations Program laboratory in Anchorage, Alaska. Nucleic acid extraction for the *S. pneumoniae* assay was by GeneJET Genomic

DNA Purification Kit (Thermo Fisher Scientific, Waltham, MA). Two hundred microliters of DNA were eluted from the original specimen and 5 µl were used in the PCR reaction. The final PCR reaction volume was 25 µl and performed by use of the TaqMan Universal Master Mix kit (Applied Biosystems, Foster City, CA) with primers and probes targeting the *lytA* gene [273]. Each run included a no-template control and a *S. pneumoniae* positive control, with DNA amplified using a Stratagene Mx3005P system (Agilent, Santa Clara, CA) with the following cycling parameters: 95°C for 10 min, followed by 40 cycles of 95°C for 15 seconds, and 60 °C for 1 minute. Amplification data were analyzed by Stratagene software and results with cycle thresholds <40 were accepted. Quantification of *S. pneumoniae* was obtained using a five-point standard curve of serial *S. pneumoniae* plasmid dilutions of known quantities ranging from 10^3 to 10^7 copies/ml. All plasmid dilutions were run in duplicate with the exception of the 10^3 dilution, which was run in triplicate.

Objective 2 Methods

The second research objective was to evaluate the impact of motavizumab on non-RSV respiratory illness during the first RSV season, and to assess the contribution of RSV and other pathogens to risk of subsequent wheeze at ages one to three years. The pneumococcal test results obtained by the methods described above were included in this analysis. In addition to the pneumococcal testing, nasopharyngeal specimens from the same events were tested for other common respiratory viruses. The viral testing was done in the laboratory of James Gern at the University of Wisconsin-Madison, in Madison, Wisconsin. For the viral multiplex PCR panel, 350ul of nasal sample was extracted using

the NucliSENS EasyMag kit (bioMerieux, Marcy l'Etoile, France) with an RNA eluate volume of 25ul. 10ul of eluate was used for the real-time NxTAG® Respiratory Pathogen Panel (Luminex Corporation, Austin, Texas). The NxTAG® panel includes influenza virus A (multiple subtypes) and B (Flu A and Flu B), RSV A and B, coronaviruses (subtypes 229E, HKU1, NL63, and OC43), human metapneumovirus (HMPV), human rhinovirus/enterovirus (HRV/EV), adenovirus, parainfluenza viruses 1-4, bocavirus, and the bacterial pathogens *Chlamydophila pneumoniae* and *Mycoplasma pneumonia*. cDNA was generated with the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) and 10ul of RNA eluate. Rhinovirus/enterovirus positive samples were typed using a molecular typing assay [274]. In instances where there was discordance between the RSV result from the NxTAG® panel and the RSV result from the testing done as part of the primary testing for the clinical trial, we deferred to the primary result.

Objective 3 Methods

The third research objective was to assess whether there was an increase in RSV disease in the second RSV season following immunoprophylaxis in the first. The second RSV season was defined as the continuous time period between the middle of October of one year until the end of May of the next year. This definition is consistent with the enrollment and follow up periods for the motavizumab parent study, where children were enrolled between October 15th and December 31st, and were followed for medically attended RSV illness for 150 days following enrollment, with the end of 150 day follow up falling between March 16th and May 30th, depending on the enrollment date. For all participants in this analysis, the second RSV season was defined as the period of time

between October 15th and May 30th in the calendar year(s) following study enrollment (Table 4.2.1). Specimens eligible for testing were those collected within five days of an inpatient or outpatient event that occurred during the second RSV season and that was determined to be a lower respiratory illness.

Table 4.2.1 Dates corresponding to RSV Season 1 enrollment for the four study cohorts

		RSV Season 1		RSV Season 2			
		Day 0 (Start)	Day 150 (End)	Start	Study day	End	Study day
Enrollment Cohort 1							
	Start Enroll[1]	11/15/04	4/14/05	10/15/05	334	5/30/06	561
	End Enroll[2]	12/30/04	5/29/05	10/15/05	289	5/30/06	516
Enrollment Cohort 2							
	Start Enroll	10/17/05	3/16/06	10/15/06	363	5/30/07	590
	End Enroll	12/30/05	5/29/06	10/15/05	289	5/30/07	516
Enrollment Cohort 3							
	Start Enroll	11/30/06	4/29/07	10/15/07	319	5/30/08	547
	End Enroll	12/31/06	5/30/07	10/15/07	288	5/30/08	516
Enrollment Cohort 4							
	Start Enroll	10/15/07	3/13/08	10/15/08	366	5/30/09	593
	End Enroll	12/31/07	5/29/08	10/15/08	289	5/30/09	516

[1]The first date of enrollment of a participant in this cohort
[2]The last date of enrollment of a participant in this cohort

Samples collected at second season events were tested for RSV using the respiratory multiplex panel described in the objective two methods section.

Analytic methods

Analytic methods are described for each objective in chapters 5-7.

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Chapter 5: Probing the interaction of respiratory syncytial virus and *Streptococcus pneumoniae* by preventing RSV lower respiratory illness with a monoclonal antibody

Abstract

Background:

Respiratory syncytial virus (RSV) and *Streptococcus pneumoniae* are common causes of lower respiratory illness (LRI) in children. A growing body of evidence suggests that these two pathogens may act synergistically in the development of LRI, with greater incidence and severity of disease occurring with both pathogens than with either alone. Recently, a phase three double blinded randomized trial of a next-generation RSV monoclonal antibody, motavizumab, showed high efficacy for the prevention of RSV associated medically attended lower respiratory illness (MALRI) in a population of healthy full term infants. We used samples collected at these MALRI events to assess whether *S. pneumoniae* nasopharyngeal carriage, a necessary precursor to the development of pneumococcal LRI, was associated with RSV MALRI and if motavizumab prophylaxis altered its prevalence and density.

Methods:

Infants less than six months of age by December 31st of any of the four years of enrollment in the motavizumab trial were enrolled and prophylaxed with monthly doses of motavizumab or placebo for five total doses through the RSV season (150 days following randomization). Nasopharyngeal secretions (washes or aspirates) were collected at every MALRI for the first 150 days following randomization and tested for

RSV. We tested stored samples from these events for *S. pneumoniae* carriage by quantitative PCR.

Results:

We found no difference in prevalence of S. *pneumoniae* carriage by RSV status (65.6% of RSV associated MALRI and 64.9% of non-RSV MALRI had *S. pneumoniae* detected, (p=0.87)), but mean carriage density was greater in RSV associated MALRIs compared to those without RSV (6.01 log(10) copies/mL vs. 5.73 log(10) copies/mL, p=0.03). The proportion of events with pneumococcal carriage density greater than 6.9 log(10) copies/mL was also greater in RSV-associated MALRI compared to non RSV MALRI (14.8% vs. 9.0%, p=0.03). There was a corresponding reduction in the density of pneumococcal carriage at MALRI events that occurred in the motavizumab treatment group compared to the placebo treatment group, although this did not reach statistical significance.

Discussion:

Our results support the hypothesis that *S. pneumoniae* and RSV may interact synergistically and suggest that preventing RSV illness with a monoclonal antibody, or potentially a vaccine, in infancy may decrease rates of high-density pneumococcal carriage in the nasopharynx. This may in turn reduce pneumococcal transmission in communities, as well as lowering the risk of development of pneumococcal disease. Future studies of RSV immunoprophylaxis products and vaccines should consider measuring rates of pneumococcal disease as outcome measures to evaluate this association.

Conclusion:

Preventing RSV lower respiratory illness in infancy likely reduces pneumococcal carriage density in the infant nasopharynx, and this may lead to a corresponding reduction in pneumococcal transmission and disease.

Introduction

Respiratory syncytial virus (RSV) is the most commonly identified virus in children with lower respiratory illness (LRI), with 33.1 million RSV-associated LRI cases in 2015, and with peak incidence occurring in infants <6 months [29]. *Streptococcus pneumoniae* (pneumococcus) is a leading cause of bacterial LRI in children under five years of age, with 3.7 million severe pneumococcal pneumonia episodes in this age group in 2015 [275]. A growing body of evidence suggests that RSV and pneumococcus may interact in lower respiratory illness in a synergistic manner. Epidemiologic studies of the association between RSV-LRI and pneumococcal pneumonia are limited, however, by difficulty in obtaining clinical specimens directly from lower respiratory tract, poor diagnostic sensitivity for bacterial pneumonia, and by the challenge of determining the true etiology of an LRI event. Colonization of the nasopharynx with *S. pneumoniae* is a necessary precursor to pneumococcal disease [276, 277], and several studies have found RSV-LRI in children to be associated with increased pneumococcal colonization density [245, 247-250, 278]. High pneumococcal carriage density in the nasopharynx has also been shown to be associated with pneumococcal pneumonia in children [249]. Vaccine probe studies offer a unique opportunity to identify causal interactions between pathogens, with pneumococcal conjugate vaccine (PCV) impact studies allowing for the quantification of the fraction of RSV-LRI that includes some vaccine-preventable pneumococcal contribution to disease [267]. In South Africa, PCV use was associated with a 32%

reduction in incidence of RSV-associated pneumonia among children <5 years, and in the Gambia it was associated with 39% reduction in incidence of all-cause bronchiolitis in infants 2-11 months [268, 270]. The inference from these studies is that the causal chain for these RSV-attributed events must include *S. pneumoniae* if they can be prevented by PCV. Experimental studies support this inference, with evidence that RSV infection preceding or simultaneous to exposure to *S. pneumoniae* increases bacterial adherence and virulence and, in animal models, leads to increased disease severity compared to RSV infection alone [193-195, 199, 205]. If RSV infection of the upper airway epithelium facilitates pneumococcal colonization and allows for increased carriage density, which then increases the risk of subsequent pneumococcal disease, we would expect that preventing RSV-illness in children would be associated with decreases in pneumococcal carriage and disease. There are currently no licensed RSV vaccines with which to evaluate this relationship, but a recent large-scale efficacy trial of a next generation anti-RSV monoclonal antibody, motavizumab, serves as a probe to assess the role of RSV-prevention on *S. pneumoniae* colonization in a population of healthy full-term infants. In this randomized, placebo-controlled trial conducted over the course of four consecutive RSV seasons, receipt of motavizumab was associated with an 87% reduction in inpatient medically attended RSV-associated LRI (RSV-MALRI) and a 71% reduction in outpatient RSV-MALRI [140]. In addition to preventing RSV-MALRI, motavizumab has been shown to reduce RSV infection of the upper airway in pre-clinical studies, although this finding has not been confirmed in humans [125]. It is conceivable that motavizumab prophylaxis could therefore prevent disease with *S. pneumoniae* by reducing pneumococcal colonization in the upper respiratory tract, or by preventing RSV

infection from progressing to lower respiratory tract illness and thereby removing the opportunity for the two pathogens to interact synergistically in the lower airways. We undertook an evaluation of the prevalence and density of *S. pneumoniae* nasopharyngeal colonization during MALRI events in this trial with the hypothesis that those who had received motavizumab and were relatively protected from RSV disease would also have lower density and prevalence of pneumococcal colonization.

Methods

Study population

The full methods of the phase 3 double-blinded placebo controlled randomized trial of motavizumab (the parent study) have been published elsewhere [140]. Briefly, healthy Native American infants living on the Navajo Nation, White Mountain Apache and San Carlos Apache Indian reservations who were born at full-term (≥36 weeks gestation) and were less than 6 months of age at the time of enrollment, were randomized to receive either motavizumab or placebo during the winter RSV season (5 monthly doses, 2:1 randomization). Four cohorts of infants were enrolled over four consecutive RSV seasons between 2004 and 2009, for a total of 2,127 participants. The current sub-study excluded the San Carlos Apache reservation participants, bringing the total number of participants to 2,088 (1,392 participants randomized to motavizumab, 696 randomized to placebo).

Evaluation of medically attended lower respiratory tract illness

Study participants were followed from the time of study enrollment through three years of age and assessed for inpatient and outpatient MALRI. Lower respiratory tract illness events were reviewed for inclusion by study investigators and were defined as a medical

diagnosis of bronchiolitis or pneumonia. In the absence of such a medical diagnosis, the occurrence of the lower respiratory illness was determined by the study investigator's review of the medical records for the presence of lower respiratory signs and symptoms including cough, retractions, ronchi, wheezing, crackles or rales, as well as associated signs or symptoms including coryza, fever and apnea.

Nasopharyngeal secretions collected within five days of the MALRI event date (hospital admission for inpatient events; doctor visit date for outpatient events) were considered to be within the analytic window and were included in the analysis. A nasopharyngeal specimen was collected at every MALRI visit. The collection of nasal wash secretions involved instilling 15 – 20 cc of Ringer's lactate solution into each nostril of a seated child with a bulb syringe and collecting it from the opposite nostril. In children who could not have a nasal wash specimen collected, a nasal aspirate was obtained by instilling 3 – 6 cc of sterile saline into the nose and withdrawing nasal mucus using a feeding tube with a suction device. One milliliter of nasopharyngeal specimen was mixed with 6 ml viral transport medium and then divided into 4 – 8 aliquots which were snap frozen immediately using liquid nitrogen or an ethanol/dry ice bath, and stored at -70°C. At facilities where snap freezing was not possible, aliquots were immediately stored at -80°C. After freezing, aliquots were shipped to central laboratories for storage. Those collected within 150 days of randomization (the RSV season) were tested for RSV A and B by PCR assay. Aliquots of untested specimen remained in storage at -80°C with continuous temperature monitoring.

Specimen testing for this sub-study

For the present sub-analysis, stored nasopharyngeal secretions from events that occurred during the first 150 days of follow up were tested for *S. pneumoniae* by a quantitative real-time PCR assay at the Centers for Disease Prevention and Control, Division of Preparedness and Emerging Investigations, Arctic Investigations Program laboratory in Anchorage, Alaska. Nucleic acid extraction for the *S. pneumoniae* assay was by GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, Waltham, MA). Two hundred microliters of DNA were eluted from the original specimen and 5 µl were used in the PCR reaction. The final PCR reaction volume was 25 µl and performed by use of the TaqMan Universal Master Mix kit (Applied Biosystems, Foster City, CA) with primers and probes targeting the *lytA* gene [273]. Each run included a no-template control and a *S. pneumoniae* positive control, with DNA amplified using a Stratagene Mx3005P system (Agilent, Santa Clara, CA) with the following cycling parameters: 95°C for 10 min, followed by 40 cycles of 95°C for 15 seconds, and 60 °C for 1 minute. Amplification data were analyzed by Stratagene software and results with cycle thresholds <40 were accepted. Quantification of *S. pneumoniae* was obtained using a five-point standard curve of serial *S. pneumoniae* plasmid dilutions of known quantities ranging from 10^3 to 10^7 copies/ml. All plasmid dilutions were run in duplicate with the exception of the 10^3 dilution, which was run in triplicate.

Statistical Analyses

Statistical analyses were performed using STATA13 (StataCorp. 2013. College Station, TX). $\chi 2$, Fisher's exact, Kruskal-Wallis and Student's t-tests were used for pairwise

comparisons. Linear and logistic regression models were used for continuous and dichotomous outcomes, respectively. P-values <0.05 were considered significant. Age was analyzed as a continuous and categorical predictor.

Ethical Approval

Informed consent for participation in this study was obtained from a parent or guardian of participants. Approval for this study was obtained from the Johns Hopkins Bloomberg School of Health IRB, the Phoenix Area Indian Health Service IRB, and the Navajo Nation IRB.

Results

Medically Attended Lower Respiratory Illness Events

During the 150 days following randomization, there were 276 respiratory hospital admissions among 231 participants (192 children with one admission; 33 children with two admissions, and 6 children with three admissions), and 653 outpatient LRI visits among 511 participants (395 children with one visit; 94 children with two visits, 18 children with three visits, and 4 children with four visits each). The mean age at inpatient events was 3.9 months while the mean age at outpatient events was 5.2 months ($p<0.001$). Nasopharyngeal secretions were collected in 264/276 (96%) of hospital admissions and 516/653 (79%) of outpatient visits. RSV test results were available for 261/276 (95%) of inpatient events and 499/653 (76%) of outpatient visits, and as reported previously in the primary efficacy analysis, both inpatient and outpatient RSV MALRI were significantly reduced in the motavizumab compared to the placebo group [140]. All

of the stored nasopharyngeal specimens that could be located (81% [223/276] of inpatient events and 68% [444/653] of outpatient events) were tested for pneumococcal carriage (Figure 5.1). We compared treatment group, RSV-status and age at event for samples with test results available for *S. pneumoniae* compared to those without. Inpatient events were more likely to be missing results for *S. pneumoniae* if they were from the motavizumab group, or if they were RSV-positive; outpatient events were also less likely to be tested for *S. pneumoniae* if they were RSV-positive, but with no difference by treatment group. There there was no difference in mean age at event for those with samples available for testing compare to those with samples not available for testing (Table 5.1).

Of 667 samples tested, *S. pneumoniae* was detected in 435 (65.2%), with no significant difference in colonization prevalence between inpatient (142/223 [63.7%]) and outpatient (293/444 [66.0%]) samples (p=0.55). Among all positive pneumococcal samples, mean density was 5.80 log(10) copies/ml (95% CI 5.70, 5.91), with mean density of 5.71 log(10) copies/ml (95% CI 5.53, 5.88) in samples from inpatient events and 5.85 log(10) copies/ml (95% CI 5.73, 5.98) in samples from outpatient events (p=0.19). The probability of pneumococcal colonization was lowest in the 0-1 month age group, increased through three months of age and then remained relatively constant, with a marginally statistically significant trend of increasing colonization by month of age (odds ratio (OR) 1.07, (95%CI 1.00, 1.15) (Figure 5.2). Mean pneumococcal density was highest in the oldest age group (10-11 months) but there was no overall statistically significant trend for increasing density with age (Kruskal-Wallis χ^2 13.8, p=0.24) (Figure

5.3). The mean age at RSV-MALRI events during the first 150 days of follow up was not different than the mean age at non-RSV viral MALRI events (3.80 mo vs. 3.81 mo, respectively, for inpatient events p=0.78), and (5.37 mo vs. 5.12 mo, respectively, for outpatient events p=0.30). After stratifying by event type (inpatient vs. outpatient), there were no statistically significant differences in mean age for children colonized with pneumococcus compared to those not colonized, by RSV-status (Figure 5.4). While detection of RSV at MALRI events showed a distinct seasonal pattern, detection of *S. pneumoniae* was consistent over time (Figure 5.5). There was no observed difference in *S. pneumoniae* detection frequency or density for samples collected up to five days prior to the event date (78/250 [68.8%]), compared to those collected on or up to five days after the event date (263/417 [63.1%]) (p=0.13).

Stratifying by RSV-status at the MALRI event, there was no statistically significant difference in prevalence of *S. pneumoniae* detection between RSV-positive and RSV-negative events, but there was a mean increase in carriage density for RSV associated MALRIs compared to those without RSV (6.01 log(10) copies/mL in RSV associated MALRI vs. 5.73 log(10) copies/mL in non RSV associated MALRI, p=0.03) (Table 5.2 and Figure 5.6). We also assessed the proportion of events with pneumococcal carriage at or above a density threshold (6.9 log(10) copies/mL), which has been shown in another population to be associated with severe pneumococcal pneumonia [249] and found that it was higher in RSV-MALRI compared to non RSV-MALRI events (14.8% vs. 9.0%, p=0.03) (Table 5.2). Adjusting for age did not alter any of these associations. Despite the associations we observed between RSV-MALRI and increased pneumococcal carriage

density, a receiver operator characteristic curve analysis did not identify a density threshold that could be used to distinguish RSV-positive from RSV-negative events in our study population (Figure 5.7).

When we stratified the analysis of RSV-MALRI and pneumococcal carriage prevalence and density by treatment group, the trends remained the same with the exception of outpatient events in the motavizumab group, where pneumococcus was significantly more likely to be detected in RSV-positive MALRIs than RSV-negative MALRIs (Table 5.3). Conversely, there was reduced pneumococcal carriage in the RSV-positive inpatient events compared to RSV negative inpatient events, though this did not reach statistical significance.

We found no difference in overall colonization prevalence by treatment group (Table 5.4). There was a trend of increased colonization density in the placebo compared to the motavizumab group, however, and a higher proportion of placebo participants had pneumococcal densities above the density threshold for pneumococcal disease, although neither of these outcomes reached statistical significance (Table 5.4).

Discussion

As previously reported, a double-blinded randomized trial of the RSV mAb motavizumab found 87% efficacy for the prevention of inpatient RSV MALRI and 71% efficacy for the prevention of outpatient RSV MALRI in a population of healthy, full-term infants who had been randomized to receive either motavizumab or placebo treatment [140]. We

tested nasopharyngeal specimens collected during these MALRI events for *S. pneumoniae* and found pneumococcal colonization of the nasopharynx to be more dense, but not more prevalent, in lower respiratory illnesses that were associated with RSV compared to those not associated with RSV. We observed a corresponding reduction in the density of pneumococcal carriage at MALRI events that occurred in the motavizumab treatment group compared to the placebo treatment group, although this did not reach statistical significance. Mean *S. pneumoniae* carriage density was approximately two-fold greater in nasopharyngeal specimens where RSV was also detected, compared to those without RSV. This is consistent with a study that found RSV-associated LRI hospitalizations to be associated with a 3-fold increase in pneumococcal density in the nasopharynx compared to RSV-negative LRI hospitalizations [244], as well other studies that similarly found an association between pneumococcal carriage density and RSV LRI [246, 248].

Pneumococcus is commonly carried in the nasopharynx of healthy children, and is a precondition for lower respiratory tract pneumococcal disease. We cannot infer from this study whether RSV lower respiratory illness is associated with pneumococcal pneumonia or whether motavizumab may have prevented pneumococcal pneumonia cases during the RSV season. Furthermore, the magnitude of the difference in mean density between samples collected at RSV LRI events compared to non-RSV LRI events may not be clinically significant. It has been demonstrated, however, that high pneumococcal carriage density is associated with microbiologically confirmed severe pneumococcal pneumonia in children [249]. We assessed the proportion of MALRI events in our study with carriage densities above a threshold associated with pneumococcal pneumonia and

found the proportion of illness events with densities above this threshold to be higher in the placebo compared to the motavizumab group, although this also did not reach statistical significance. This suggests that there may be a potential role for RSV prevention in the reduction of pneumococcal disease episodes, and that this relationship merits further investigation. Furthermore, pneumococcal colonization density likely plays a role in pneumococcal transmission, and lowering the bacterial load of pneumococcus in the nasopharynx could have important implications for control of the pathogen in the community.

An exception to the overall trend we observed was that within the motavizumab treatment group, *S. pneumoniae* carriage prevalence was significantly higher in outpatient RSV MALRI events, compared to outpatient events not associated with RSV. We hypothesize that some of the prophylaxed infants who had RSV detected at an outpatient lower respiratory illness may have had LRI that was causally associated with pneumococcus rather than RSV. Paradoxically, pneumococcus was less likely to be co-detected with RSV among inpatient events in the motavizumab group than with non-RSV inpatient events. This is counter to our expectation that pneumococcus would be more frequently detected in RSV-positive specimens, and was not consistent with what we observed in the placebo arm.

Our study did not find a difference in *S. pneumoniae* carriage frequency or density by disease severity using inpatient/outpatient event status as an indicator of severity. Other studies of infants hospitalized with RSV respiratory illness have shown mixed results regarding the association between pneumococcal carriage and clinical severity in children hospitalized with RSV LRI [74, 165, 245, 246].

We are unable to infer from the current study whether RSV infection preceded an increase in pneumococcal density, or whether infants with high pneumococcal density were more likely to develop RSV LRI, but the available evidence demonstrates several mechanisms whereby preceding RSV infection facilitates increased bacterial adherence and virulence in a way that could allow for increased density [199, 205]. There is also evidence from the United States, as well as other temperate climate settings, of a temporal relationship with peaks in invasive pneumococcal disease following RSV seasonal epidemics [90].

A limitation of our study was our reliance on available nasopharyngeal specimens. RSV-positive samples and inpatient samples from the motavizumab group were less likely to be available for testing, and while we are unable to construct an obvious scenario where this would produce bias in our results, it is possible that one exists. There may have been a difference in the density of pneumococcus detected in nasal wash compared to nasal aspirate samples, but sample type did not differ by treatment group, so is not likely to influence our overall interpretation of the results. Our study's power to detect statistically significant associations was limited by the number of events in the parent trial and the number of specimens that were collected in the analytic window and available for additional testing.

To our knowledge, this is the first time that the impact of RSV immunoprophylaxis on pneumococcal carriage and density has been assessed. An extension of the current study would be to evaluate the distribution of pneumococcal serotypes detected among RSV-associated compared to non-RSV respiratory events. At least one previous study has shown RSV infection to be associated with non-invasive pneumococcal serotypes, but the

finding was only marginally statistically significant and the study population was restricted to children with radiographically confirmed pneumonia [251]. *S. pneumoniae* density has also been shown to vary by serotype [279], and the difference in colonization densities we observed between RSV-associated and non-RSV-associated events could in part be driven by differential serotype distribution between these two groups.

As promising RSV vaccine and long-acting monoclonal antibody candidates continue to progress through clinical trials, the likelihood of a licensed product for the prevention of RSV illness in the general infant population grows closer. A better understanding of how the prevention of RSV illness may lead to reductions in the transmission of S. *pneumoniae* and potentially prevent pneumococcal disease not just in vaccinated infants but also in their communities will be an important component of the investment case for such an intervention, particularly in settings of high pneumococcal pneumonia burden.

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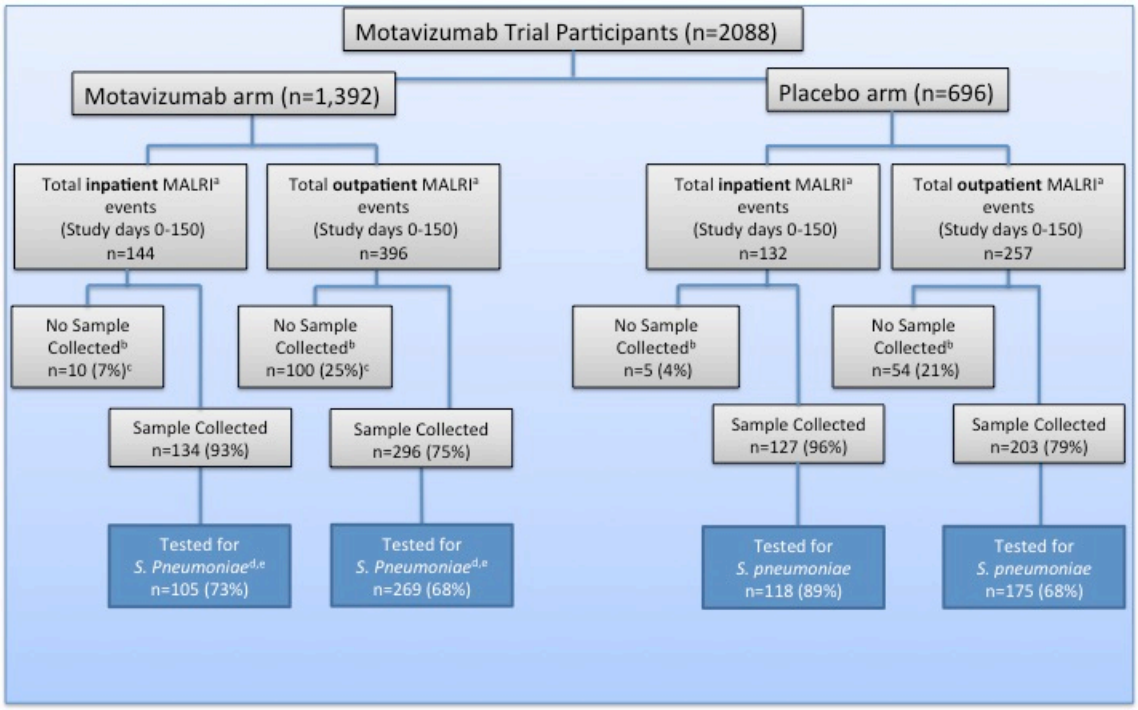
Tables and Figures

Figure 5.1 Nasopharyngeal specimens tested for *Streptococcus pneumoniae*



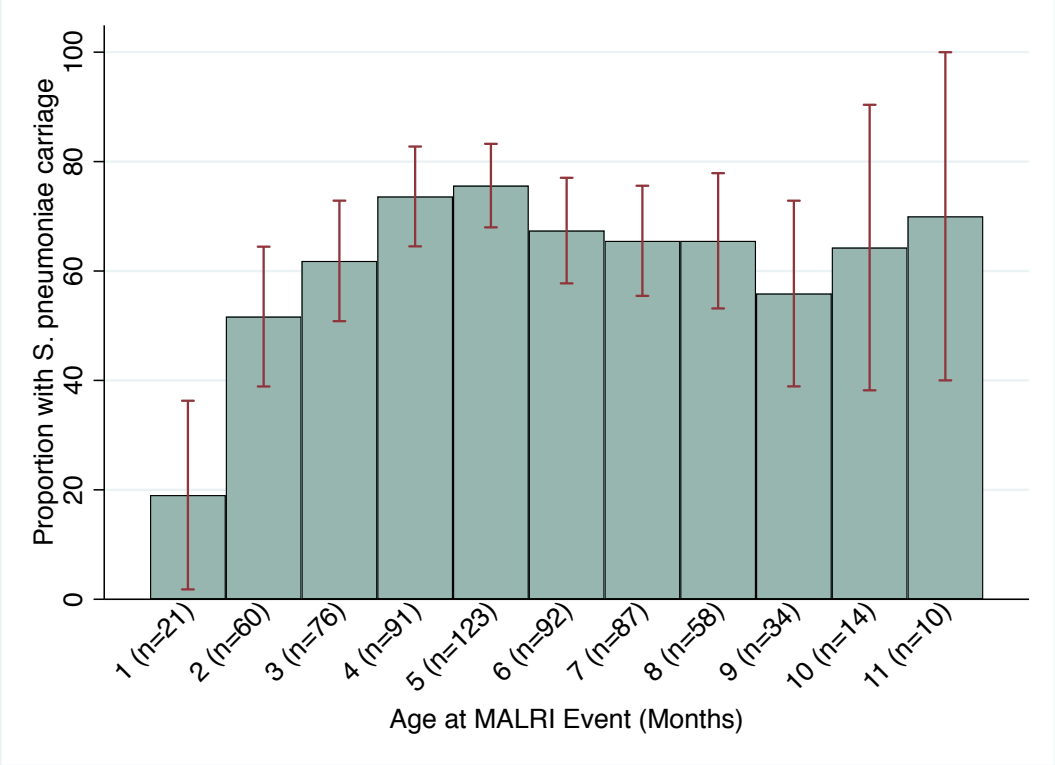
Key:
[a]medically attended lower respiratory illness
[b]within 5 days of event (analytic window)
[C]denominator for sample collection and testing is total number of events
[d]*Streptococcus pneumoniae*
[e]denominator for results is total number tested

Table 5.1 Characteristics of MALRI[1] samples according to testing for *S. pneumoniae*, by treatment group and RSV status

	Age		Treatment Group			RSV Status[2]		
	Mean age, mo (SD)	p-value[3]	Mota n (%)	Placebo n (%)	p-value[4]	RSV pos n (%)	RSV neg n (%)	p-value[4]
Inpatient Events								
			n=136	**n=128**		**n=105**	**n=156**	
Tested	3.8 (2.2)	0.52	105 (77.2)	118 (92.2)	**<0.01**	98 (93.3)	125 (80.1)	**<0.01**
Not Tested	4.1 (2.4)		31 (22.8)	10 (7.8)		7 (6.7)	31 (19.9)	
Outpatient Events								
			n=309	**n=207**		**n=109**	**n=383**	
Tested	5.3 (2.1)	0.40	269 (87.1)	175 (84.5)	0.42	91 (83.5)	351 (91.6)	**0.01**
Not Tested	5.0 (2.4)		40 (12.9)	32 (15.5)		18 (16.5)	32 (8.4)	
All Events								
			n=445	**n=335**		**n=214**	**n=539**	
Tested	4.8 (2.3)	0.67	374 (84.0)	293 (87.5)	0.18	189 (88.3)	476 (88.3)	0.99
Not Tested	4.7 (2.4)		71 (16.0)	42 (12.5)		25 (11.7)	63 (11.7)	

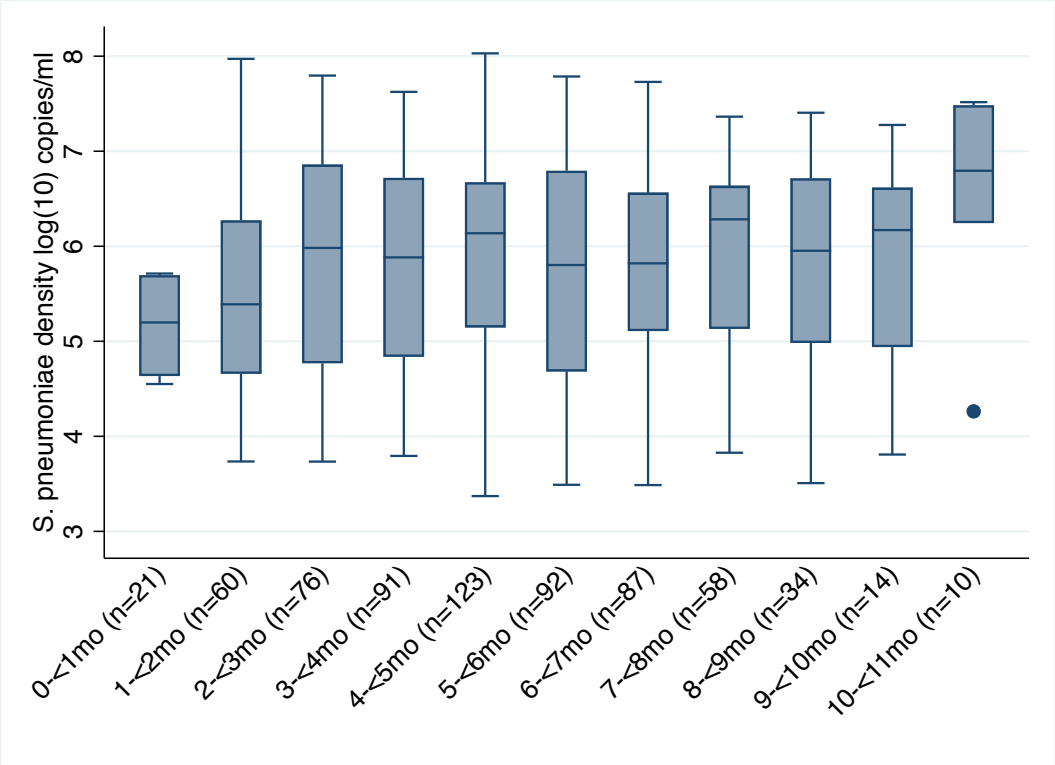
[1]Medically attended lower respiratory illness
[2]Three inpatient events and 24 outpatient events have no RSV result in the database, though NP specimens were indicated as collected; 0/3 of the inpatient events had samples located and tested for *S. pneumoniae;* 2/24 outpatient events had samples located and tested for *S. pneumoniae* – one was collected 1 day after event date; the other was collected 6 days prior to the event date.
[3]Student's t-test
[4]χ^2 test

Figure 5.2 Proportion colonized with *S. pneumoniae* at MALRI[1] event, by age with 95% confidence intervals



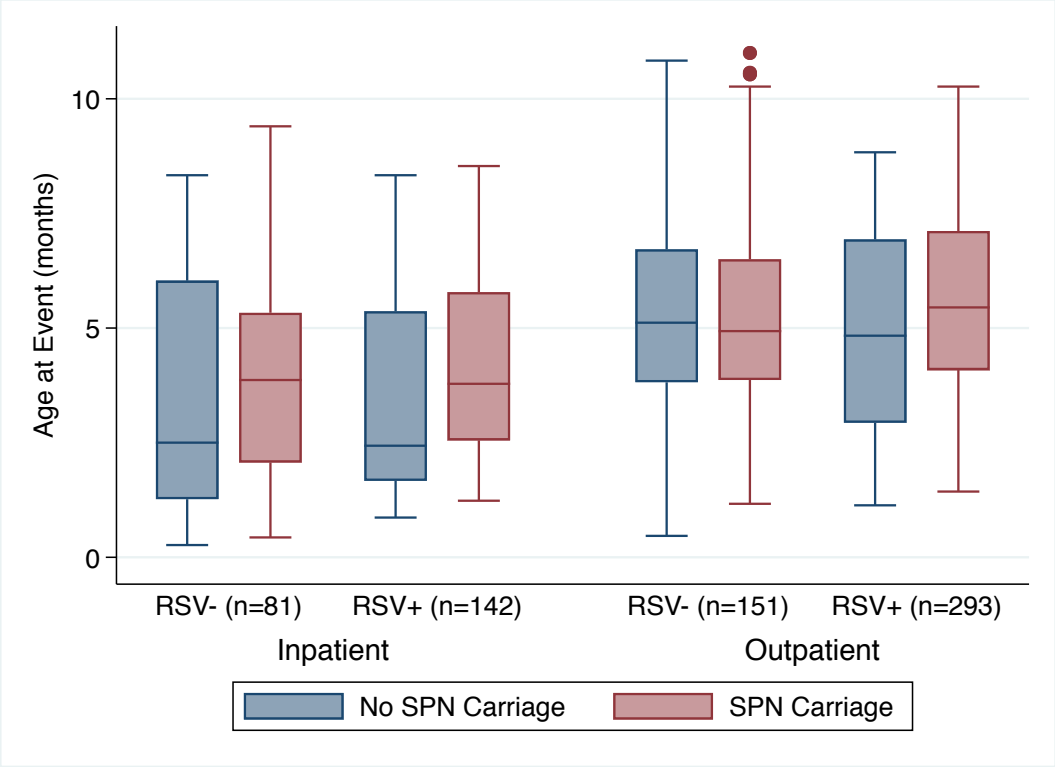
[1]Medically attended lower respiratory illness

Figure 5.3 Mean *S. pneumoniae* colonization density at MALRI[1] event, by age



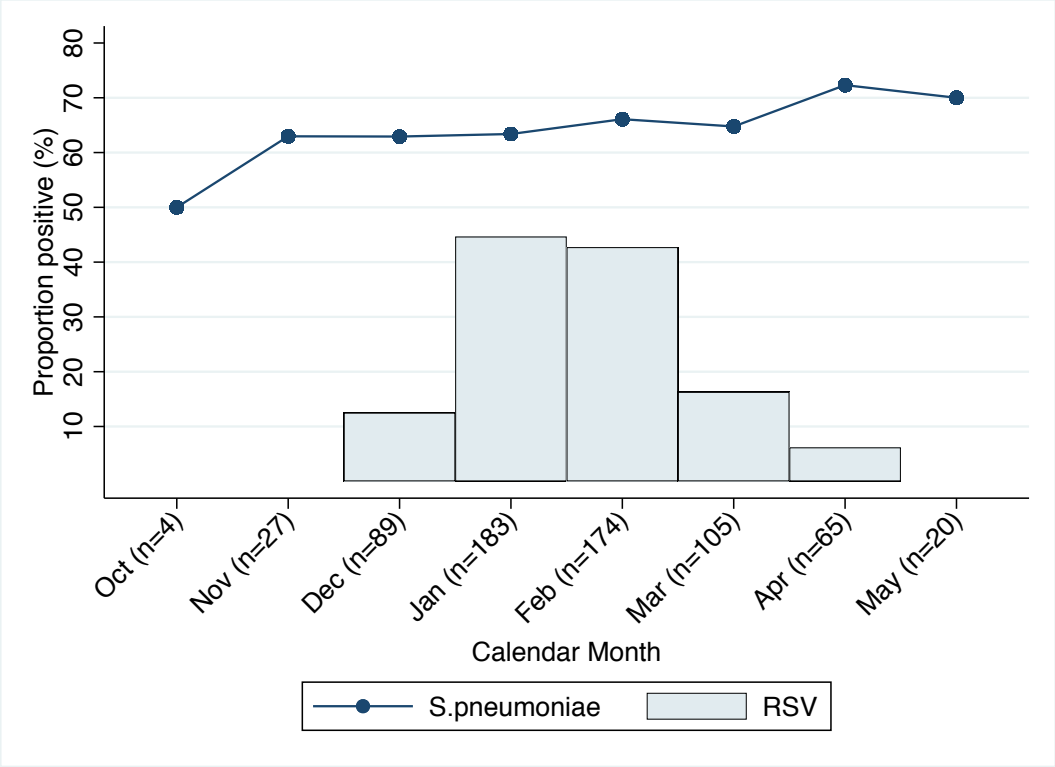
[1]Medically attended lower respiratory illness

Figure 5.4 Age distribution of participants with *S. pneumoniae* Carriage at MALRI[1] event, by RSV status



[1]Medically attended lower respiratory illness

Figure 5.5 Frequency of RSV and *S. pneumoniae* detection at MALRI[1] events by calendar month



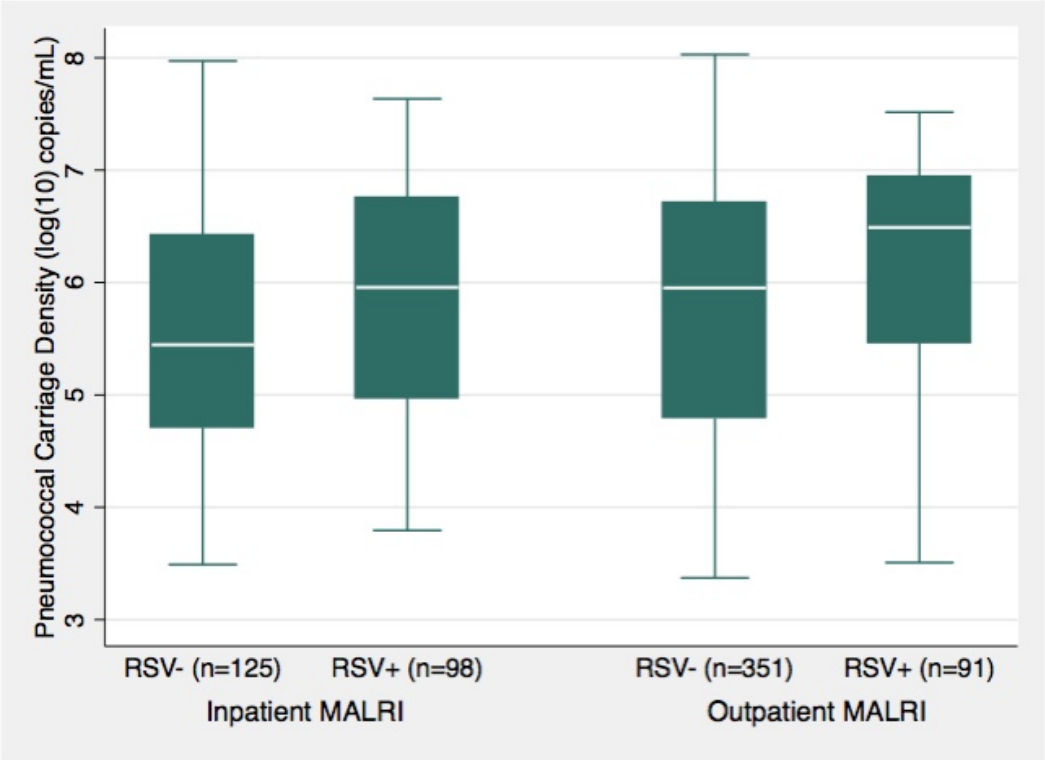
[1]Medically attended lower respiratory illness

Table 5.2 Association between RSV-associated medically attended lower respiratory illness and colonization with *Streptococcus pneumoniae*

	RSV status[1]	Spn Col. N (%)	OR (95% CI)	Age-adjusted OR (95% CI)	p-value[3]	Col. above threshold	OR (95% CI)	p-value[3]	Mean density Log_{10} copies/mL (SD)	p-value[4]
Inpatient MALRI[2]										
	RSV-positive (n=98)	60 (61.2)	0.83 (0.48, 1.43)	0.81 (0.46, 1.41)	0.50	11 (11.2)	2.13 (0.82, 5.55)	0.13	5.90 (1.03)	0.06
	RSV-negative (n=125)	82 (65.6)				7 (5.6)			5.56 (1.05)	
Outpatient MALRI										
	RSV-positive (n=91)	64 (70.3)	1.29 (0.79, 2.13)	1.29 (0.78, 2.12)	0.31	17 (18.7)	2.01 (1.08, 3.75)	**0.03**	6.12 (1.07)	**0.03**
	RSV-negative (n=351)	227 (64.7)				36 (10.3)			5.78 (1.12)	
Any MALRI										
	RSV-positive (n=189)	124 (65.6)	1.03 (0.72, 1.47)	1.05 (0.73, 1.49)	0.87	28 (14.8)	1.75 (1.06, 2.90)	**0.03**	6.01 (1.05)	**0.01**
	RSV-negative (n=476)	309 (64.9)				43 (9.0)			5.73 (1.10)	

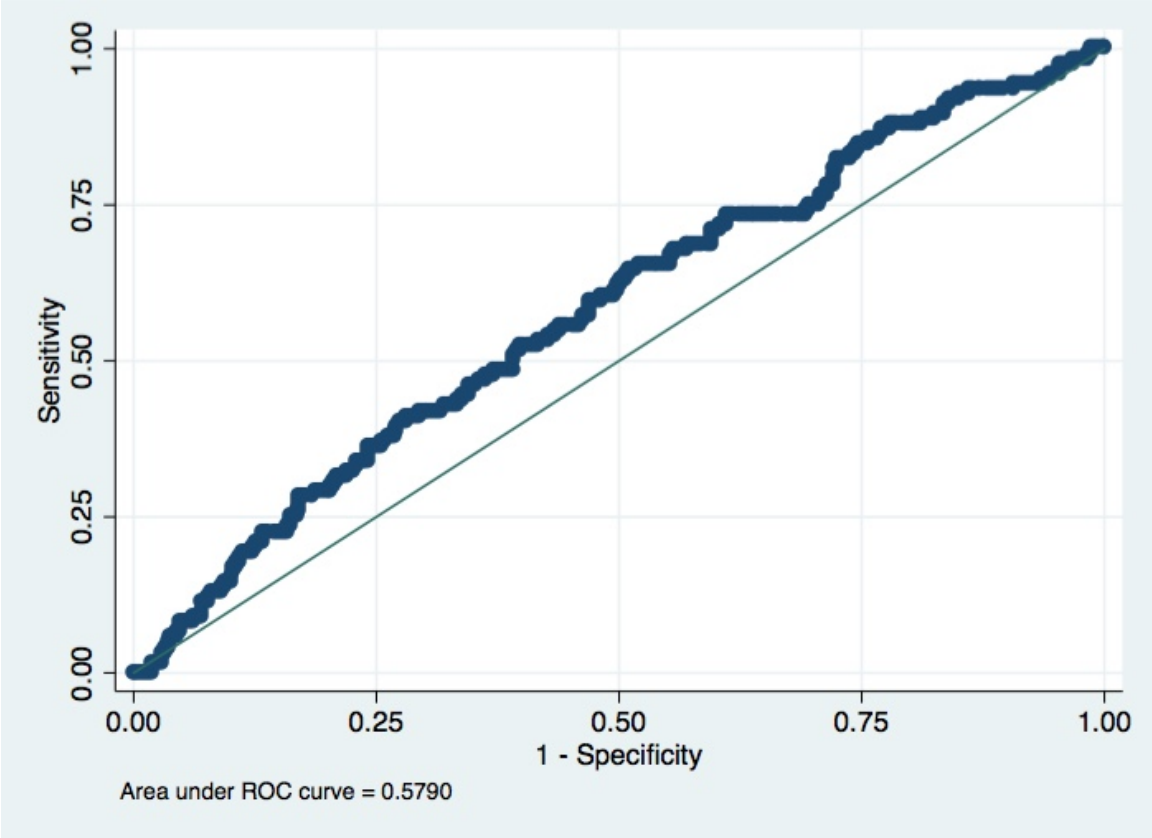
[1]Denominators include events with specimen collected and tested for pneumococcal carriage
[2]Medically attended lower respiratory illness
[3]χ^2 test
[4]student's t-test

Figure 5.6 Pneumococcal carriage density by RSV-MALRI[1] status



[1] RSV-associated medically attended lower respiratory illness

Figure 5.7 Receiver Operator Characteristic Analysis of *Streptococcus pneumoniae* colonization density to distinguish RSV-positive from RSV-negative MALRI[1] events



[1]Medically attended lower respiratory illness

Table 5.3 Association between RSV-associated medically attended lower respiratory illness and colonization with *Streptococcus pneumoniae*, stratified by treatment group

	Treatment Group[1]	Pneumococcal Colonization N (%)	p-value[2]	Proportion with density above threshold N(%)	p-value[2]	Mean density Log_{10} copies/mL (SD)	p-value[3]
Motavizumab							
Inpatient							
	RSV-Positive (n=19)	9 (47.4)	0.15	1 (5.2)	0.91	5.71 (1.05)	0.57
	RSV-Negative (n=86)	56 (65.1)		4 (4.7)		5.50 (1.08)	
Outpatient							
	RSV-Positive (n=33)	6 (81.2)	**0.04**	5 (15.1)	0.44	5.88 (1.13)	0.76
	RSV-Negative (n=235)	150 (63.8)		25 (10.6)		5.81 (1.12)	
Placebo							
Inpatient	RSV-Positive (n=79)	51 (64.6)	0.82	10 (12.7)	0.42	5.93 (1.03)	0.37
	RSV-Negative (n=39)	26 (66.7)		3 (7.7)		5.71 (0.99)	
Outpatient							
	RSV-Positive (n=58)	37 (63.8)	0.73	12 (20.7)	**0.04**	6.29 (1.00)	**0.01**
	RSV-Negative (n=116)	77 (66.4)		11 (9.5)		5.73 (1.12)	

[1]Denominators include events with specimen collected and tested for pneumococcal carriage
[2]χ^2 test
[3]student's t-test

Table 5.4 Association between treatment group and colonization with *Streptococcus pneumoniae* at medically attended lower respiratory illness events

	Treatment Group[1]	Pneumococcal Colonization N (%)	p-value[2]	Proportion with density above threshold N(%)	p-value[2]	Mean density Log_{10} copies/mL (SD)	p-value[3]
Inpatient MALRI							
	Motavizumab (n=105)	65 (61.9)	0.60	5 (4.8)	0.09	5.53 (1.07)	0.06
	Placebo (n=118)	77 (65.3)		13 (11.0)		5.86 (1.01)	
Outpatient MALRI							
	Motavizumab (n=269)	178 (66.2)	0.92	30 (11.12)	0.53	5.81 (1.12)	0.43
	Placebo (n=175)	115 (65.7)		23 (13.1)		5.92 (1.11)	
Any MALRI							
	Motavizumab (n=374)	243 (65.0)	0.88	35 (9.4)	0.22	5.74 (5.60)	0.14
	Placebo (n=293)	192 (65.8)		36 (12.3)		5.89 (1.07)	

[1]Denominators include events with specimen collected and tested for pneumococcal carriage
[2]χ^2 test
[3]student's t-test

Chapter 6: The impact of preventing respiratory syncytial virus lower respiratory illness on other respiratory pathogens and on subsequent medically attended wheeze

Abstract

Background: A double-blinded placebo controlled randomized trial of motavizumab in healthy, full term infants reported high efficacy against respiratory syncytial virus (RSV) inpatient and outpatient medically attended lower respiratory illness (MALRI), but no efficacy against subsequent medically attended wheezing through 3 years of age. We evaluated the risk of non-RSV MALRI and the basis for the lack of efficacy against subsequent wheezing.

Methods: Infants less than six months of age by December 31st of any of the four years of enrollment in the motavizumab trial were enrolled and prophylaxed with monthly doses of motavizumab or placebo for five total doses through the RSV season (150 days following randomization). We tested stored nasopharyngeal specimens from MALRIs occurring during the first 150 days of the trial follow up period by viral multiplex and *Streptococcus pneumoniae* PCR. Human rhinovirus (HRV) positive samples were subtyped. We evaluated these and other exposures for medically attended wheeze at ages 1-3 years of age (subsequent wheeze).

Results: Motavizumab reduced MALRI with RSV alone and in the presence of other viruses in inpatients [RR 0.13 (95%CI: 0.06, 0.24) and RR 0.12 (95%CI 0.05, 0.25), respectively], and in outpatients [RR 0.15 (95%CI 0.07, 0.30) and RR 0.48 95%CI (0.30, 0.79), respectively]. Rates of outpatient influenza type A (Flu A) and human metapneumovirus (HMPV) associated MALRIs were increased in motavizumab compared to placebo participants. A family history of asthma, exposure to children in

daycare, and MALRI with HRV subtype A and C, parainfluenza virus (PIV), or coronavirus during the first 150 days of follow up, were independently associated with subsequent wheeze at ages 1-3 years. RSV (inpatient) MALRI was associated with subsequent wheeze in the motavizumab group (i.e. children who broke through motavizumab prophylaxis) but not the placebo group.

Discussion: Motavizumab prevents MALRI with RSV alone and in combination with other viruses. The comparatively reduced efficacy for outpatient RSV MALRI with other viruses suggests that that as disease severity is reduced, RSV test-positivity becomes less specific for LRI causality, which has implications for future efficacy trials. RSV MALRI was not independently associated with subsequent medically attended wheeze at ages 1-3 years in this study population, but PIV, coronavirus and HRV MALRIs were. Participants who experienced motavizumab break through were at significantly increased risk of subsequent wheeze after adjusting for other risk factors, and may represent a subgroup of children at high risk both for severe disease given RSV infection and for subsequent wheeze, regardless of RSV-illness exposure.

Conclusion: Globally, RSV is associated with significant child morbidity, particularly in early infancy, but the role that these severe illness episodes play in the causal pathway to wheeze and asthma in later childhood is unclear. We found that motavizumab prevents MALRI with RSV alone as well as MALRI with RSV in combination with other viruses, and that certain non-RSV viral MALRIs in infancy, particularly rhinovirus, PIV and coronaviruses, may increase the risk of subsequent wheezing in some settings. Additionally, there may be a subgroup of children at high risk for both

immunoprophylaxis failure and risk of wheezing in early childhood, and this merits further investigation.

Introduction

Respiratory syncytial virus (RSV) is a leading cause of child morbidity and mortality globally, with approximately 3.2 million annual hospital admissions and 59,600 – 118,200 deaths in children less than five years of age [29]. The greatest burden of severe RSV disease and mortality occurs in infants less than six months of age [29]. An anti-RSV monoclonal antibody (mAb), palivizumab, is effective for the prevention of severe disease in high-risk infants and is licensed for use in many countries. No RSV vaccines or mAbs are currently available for use in the general population [95]. Recently, a double-blinded randomized trial of a next-generation RSV mAb, motavizumab, found high efficacy for the reduction of RSV-associated medically attended lower respiratory illness (MALRI) in healthy full-term infants [140]. This product was designed to have increased affinity and enhanced efficacy compared to the licensed first-generation mAb, palivizumab. The rate of all-cause MALRI was also significantly reduced in the motavizumab treatment group, although not by the same magnitude. A slight (2.5%, not statistically significant) increase in the absolute rate of non-RSV MALRIs in the motavizumab compared to the placebo group signaled potential antagonism between RSV and other pathogens, whereby the reduction in the presence of RSV may have allowed for increased presence by these other pathogens [140]. Potential antagonism between RSV and other pathogens was previously observed in a randomized controlled trial of palivizumab mAb in preterm infants where there was no overall reduction in all-cause MALRI despite a significant reduction in RSV MALRI [175]. A negative association between detection of RSV and human rhinovirus in infants has also been

observed in a prospective cohort study [280]. In the motavizumab study that makes up the basis for this research, samples were not tested for non-RSV respiratory pathogens.

Participants in the motavizumab trial continued to be followed for medically attended subsequent wheeze, with a finding of no difference by treatment group for wheezing between 1-3 years of age by any of the case definitions developed before un-blinding [140]. This contrasts with several, mostly observational, studies that have reported an association between severe RSV disease in infancy and increased risk of wheeze and asthma in later childhood [2, 175-178, 281]. Whether RSV disease is indeed causally associated with subsequent wheeze and asthma or whether it is merely more likely to occur in children who are predisposed to these conditions is unclear and a topic of considerable debate. There are proposed mechanisms to explain how acute RSV illness may play a causative role in long-term wheezing episodes. These include chronic epithelial and airway reactivity changes to the developing infant lung, lung injury that alters lung function, and immunomodulatory changes [2]. Against a causal association are the identification of genes that are associated with increased risk of both severe RSV illness and asthma, as well as reduced lung function at birth, which is also associated with both conditions [71, 171]. Human rhinovirus (HRV) is another virus that may increase risk of subsequent wheeze and childhood asthma, and in several studies has been shown to be a greater predictor of these outcomes than RSV illness [136]. Furthermore, in the nasopharyngeal microbiome, early asymptomatic colonization with *Streptococcus pneumoniae* has also been shown to be a strong risk factor for asthma, where bacterial co-colonization with viral infection of the upper airways is associated with the spread of

virus to the lower airways and subsequent inflammatory responses that may contribute to risk of asthma development [165]. A better understanding of the specific role of RSV disease in the development of long-term respiratory sequelae, as well as an understanding of how RSV disease prevention in infancy may modify the risk of acute illness with other respiratory pathogens, is essential for estimating the potential value of future RSV passive and active vaccination programs [93].

In this study, we hypothesized that no impact of RSV prevention on wheeze was observed because there were other viruses causing acute lower respiratory illness which were themselves associated with subsequent wheeze, and that the frequency of these MALRI events were the same or more common in the motavizumab group than the placebo group (the latter scenario being explained by antagonism between RSV and other pathogens). To address this hypothesis, we used the prior double-blind randomized trial of motavizumab in Native American children [3] to assess the following *a priori* questions: (1) whether motavizumab was equally effective in preventing RSV MALRI with and without other viral co-infections in the first 150 days following randomization, (2) whether increased rates of MALRI with non-RSV viruses occurred as a result of motavizumab prophylaxis in the first 150 days following randomization (3) the manner in which RSV and other respiratory pathogens contribute to the risk of subsequent medically attended wheeze in this study population, and (4) how infants in the motavizumab arm who were hospitalized with RSV lower respiratory illness (i.e. children with breakthrough disease) differ from infants in the motavizumab arm who did not have breakthrough disease.

Methods

Study population

The full methods of the phase 3 double-blind placebo-controlled randomized trial of motavizumab (the parent study) have been published elsewhere [140]. Briefly, healthy Native American infants living on the Navajo Nation, White Mountain Apache and San Carlos Apache Indian reservations who were born at full-term (≥36 weeks gestation) and were less than six months of age by December 31st of any of the four years of enrollment were randomized to receive either motavizumab or placebo during the winter RSV season (5 monthly doses, 2:1 randomization). Four cohorts of infants were enrolled over four consecutive RSV seasons between 2004 and 2009, for a total of 2,127 participants. The current sub-analysis excluded the San Carlos Apache reservation participants, bringing the total number of participants to 2,088 (1,392 participants randomized to motavizumab, 696 randomized to placebo).

Evaluation of medically attended lower respiratory tract illness

Study participants were followed from the time of study enrollment through three years of age and assessed for inpatient and outpatient medically attended lower respiratory tract illness (MALRI). Lower respiratory tract illness events were reviewed for inclusion by study investigators and were defined as a medical diagnosis of bronchiolitis or pneumonia. In the absence of such a medical diagnosis, the occurrence of the lower respiratory illness was determined by the study investigator's review of the medical records for the presence of lower respiratory signs and symptoms including cough,

retractions, ronchi, wheezing, crackles or rales, as well as associated signs or symptoms including coryza, fever and apnea.

Nasopharyngeal secretions collected within five days of the MALRI event date (hospital admission for inpatient events; doctor visit date for outpatient events) were considered to be within the analytic window and were included in the analysis. A nasopharyngeal specimen was collected at every MALRI visit. The collection of nasal wash secretions involved instilling 15 – 20 cc of Ringer's lactate solution into each nostril of a seated child with a bulb syringe and collecting it from the opposite nostril. In children who could not have a nasal wash specimen collected, a nasal aspirate was obtained by instilling 3 – 6 cc of sterile saline into the nose and withdrawing nasal mucus using a feeding tube with a suction device. One milliliter of nasopharyngeal specimen was mixed with 6 ml viral transport medium and then divided into 4 – 8 aliquots which were snap frozen immediately using liquid nitrogen or an ethanol/dry ice bath, and stored at -70°C. At facilities where snap freezing was not possible, aliquots were immediately stored at -80°C. After freezing, aliquots were shipped to central laboratories for storage. Those collected within 150 days of randomization (the RSV season) were tested for RSV A and B by PCR assay. Aliquots of untested specimen remained in storage at -80°C with continuous temperature monitoring.

Evaluation of wheeze

Participants were followed from study day 0 (day of first study drug) through three years of age for the occurrence of medically attended wheezing events. Medically attended wheezing was counted as an outcome event if there was a discharge diagnosis of asthma, bronchiolitis, reactive airway disease, or documentation of wheezing in the medical record by the treating physician. A new wheezing episode was defined as one that occurred more than two weeks after the diagnosis of the previous episode and did not represent a persistence of the previous episode according to medical opinion. Only new wheezing episodes were included in the wheezing analyses.

Subsequent wheeze events were those medically attended wheeze events occurring between one and three years of age, and were classified using three outcome definitions: (1) ≥1 medically attended wheeze event, (2) serious early childhood wheeze, and (3) recurrent wheeze. Serious early childhood wheeze and recurrent wheeze are two overlapping but distinct subsets of medically attended wheeze. A child was considered to have serious early childhood wheeze if s/he met any one of four conditions between one and three years of age: (1) three or more medically attended wheezing events during any 12-month period, (2) a need for one or more courses of systemic steroids for treatment of a medically attended wheezing event, (3) a need for asthma control medications over a 12-month period for at least three consecutive months (i.e., ≥90 days) or five cumulative months (i.e. ≥150 days), with duration assessed by a combination of parental interviews and medical records, or (4) a least one inpatient wheezing event. Recurrent medically attended wheeze was defined as three or more medically attended wheezing events during any 12-month period between one and three years of age.

Specimen testing for this sub-analysis

For the present sub-analysis, stored nasopharyngeal secretions from events that occurred during the first 150 days of follow up were tested by a real-time PCR multiplex viral panel and by a *S. pneumoniae* uniplex PCR assay. For the multiplex panel, 350ul of nasal sample was extracted using the NucliSENS EasyMag kit (bioMerieux, Marcy l'Etoile, France) with an RNA eluate volume of 25ul. 10ul of eluate was used for the real-time NxTAG® Respiratory Pathogen Panel (Luminex Corporation, Austin, Texas). The NxTAG® panel includes influenza virus A (multiple subtypes) and B (Flu A and Flu B), RSV A and B, coronaviruses (subtypes 229E, HKU1, NL63, and OC43), human metapneumovirus (HMPV), human rhinovirus/enterovirus (HRV/EV), adenovirus, parainfluenza viruses 1-4, bocavirus, and the bacterial pathogens *Chlamydophila pneumoniae* and *Mycoplasma pneumonia*. cDNA was generated with the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) and 10ul of RNA eluate. Rhinovirus/enterovirus positive samples were typed using a molecular typing assay [274]. In instances where there was discordance between the RSV result from the NxTAG® panel and the RSV result from the testing done as part of the primary testing for the clinical trial, we deferred to the primary result. Nucleic acid extraction for the *S. pneumoniae* assay was by GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, Waltham, MA). Two hundred microliters of DNA were eluted from the original specimen and 5 µl were used in the PCR reaction. The final PCR reaction volume was 25 µl and performed by use of the TaqMan Universal Master Mix kit (Applied Biosystems, Foster City, CA) with primers and probes targeting the *lytA* gene

[273]. Each run included a no-template control and a *S. pneumoniae* positive control, with DNA amplified using a Stratagene Mx3005P system (Agilent, Santa Clara, CA) with the following cycling parameters: 95°C for 10 min, followed by 40 cycles of 95°C for 15 seconds, and 60 °C for 1 minute. Amplification data were analyzed by Stratagene software and results with cycle thresholds <40 were accepted.

Statistical Analyses

Rates of MALRI with RSV and other viruses in the motavizumab and placebo treatment arms were calculated using the intention to treat study population of the respective treatment arm as the denominator. Relative risks for MALRI with RSV and other viruses were calculated using these rates and were then used to calculate efficacy estimates for motavizumab. Proportions of MALRI events positive for a pathogen were calculated using denominators that included only those MALRI events with a specimen tested for the pathogen of interest. Binary outcomes were compared using Fisher's exact test. Multiple logistic regression was used for adjusted analyses. Backwards, stepwise selection with alpha equal to 0.05 was used to select the final adjusted models. All p-values are two-sided. Data were analyzed using STATA (Version 13).

Ethical Approval

Informed consent for participation in this study was obtained from a parent or guardian of participants. Approval for this study was obtained from the Phoenix Area Indian Health Service IRB, and the Navajo Nation IRB, and the Johns Hopkins Bloomberg School of Health IRB.

Results

During the 150 days following randomization (first 150 days of follow up), 276 respiratory hospital admissions (inpatient MALRI events) occurred among 231 participants: 192 (83%) infants with one admission, 33 (14%) infants with two admissions, and 6 (3%) infants with three admissions. Within the same time period, there were 653 outpatient visits for lower respiratory illness (outpatient MALRI events) among 511 participants: 395 (77%) children with one visit, 94 (18%) children with two visits, 18 (4%) children with three visits, and 4 (<1%) children with four visits each. A total of 705 stored nasopharyngeal specimens were located and tested for the presence of other respiratory pathogens (76% of 929 MALRI events) (Figure 6.1.A and Figure 6.1.B). There was agreement between the original RSV result from the trial and the RSV result from the Luminex multiplex panel for 654/702 (93.2%) specimens; where the results differed we deferred to the original result. We compared treatment group, RSV infection status and age at event for samples with test results available for non-RSV pathogens compared to those without. Among inpatient events, placebo recipients were more likely than motavizumab recipients to have results for non-RSV pathogens, and RSV-positive

events were more likely to have non-RSV testing completed than RSV-negative events; outpatient events were equally likely to have complete test results regardless of treatment group, RSV status or age at event (Table 6.1).

Consistent with the primary efficacy analysis, there was a lower rate of any RSV MALRI in the motavizumab group compared to the placebo group in the subset of participants included in our analysis [4% (61/1392) vs. 22% (151/696), respectively] [140]. We stratified RSV MALRI events in the first 150 days of follow up into those with RSV detected alone and RSV detected in combination with another virus, and found efficacy for motavizumab for the prevention of both (Figure 6.2). The efficacy of motavizumab for prevention of inpatient MALRI with RSV alone was the same as that for RSV with another virus, while the efficacy for prevention of outpatient MALRI with RSV alone was greater than for RSV detected in the presence of another virus. Only RSV events that were also tested for other viruses were included in the stratified analysis of motavizumab efficacy. Three RSV-positive inpatient samples from two participants in the motavizumab arm were unavailable for additional viral testing, while none of the outpatient samples unavailable for additional viral testing from the motavizumab arm were positive for RSV. Because inpatient samples from the motavizumab group were less likely to be available for testing for other viruses than were the inpatient samples from the placebo group, we did a sensitivity analysis assigning the two motavizumab participants with RSV inpatient MALRI events not tested for other viruses to the 'RSV plus other virus' group. The subsequent re-calculated relative risk for inpatient MALRI with RSV plus another virus in the motavizumab compared to the placebo group was

0.15 (95% CI 0.07, 0.30), which did not change our original interpretation of the efficacy of motavizumab for the prevention of inpatient MALRI with RSV along with other viruses. As expected, efficacy was not demonstrated for prevention of inpatient or outpatient events associated with only non-RSV viruses (Figure 6.2).

While there was no difference by treatment group in the overall frequency of non-RSV viral MALRI events, some differences were noted when results were stratified by pathogen (Table 6.2.A and Table 6.2.B). Participants in the placebo group were more likely to have inpatient or outpatient MALRI with rhinovirus type A, and inpatient MALRI with rhinovirus type B, parainfluenza viruses or coronaviruses than motavizumab participants. However, after excluding instances of co-infection with RSV, the distribution of these viruses was the same in both treatment groups (Table 6.2.A and Table 6.2.B). The rate of outpatient MALRI with human metapneumovirus or influenza type A, however, was higher in the motavizumab group compared to the placebo group (Table 6.2.B).

At least one virus was detected in 91% (212/232) of inpatient MALRI events, and in 88% (415/473) of outpatient MALRI events occurring in the first 150 days of follow up with specimens available for testing (Figure 6.3.A and 6.3.B). The most common viruses detected across all events were HRV (40%, 284/705) and RSV (29%, 207/705). HRV predominated among all MALRI events with the exception of inpatient MALRI in the placebo group, where RSV was the predominant pathogen. With the exception of RSV, no viruses were more commonly detected in nasopharyngeal samples from the placebo

group compared to the motavizumab group. Some viruses were more commonly detected in nasopharyngeal samples from the motavizumab group compared to the placebo group, but only among outpatient events [coronavirus (50/280 (17.9%) vs. 13/193 (6.7%), p<0.001; human metapneumovirus (35/280 (12.5%) vs. 11/193 (5.7%), p=0.02; influenza type A (24/280 (8.6%) vs. 5/193 (2.6%), p=0.01; and parainfluenza viruses 40/280 (14.3%) vs. 15/193 (7.8%), p=0.04] (Figure 6.3.B).

The mean age was the same for RSV MALRI and non-RSV MALRIs during the first 150 days of follow up (3.80 mo vs. 3.81 mo, respectively, for inpatient events p=0.78), and (5.37 mo vs. 5.12 mo, respectively, for outpatient events p=0.30). Among those tested for other viruses, the proportion of inpatient RSV-positive specimens with another virus detected did not differ by treatment group (42% [8/19] in the motavizumab group vs. 44% [35/80] in the placebo group, respectively (p=1.00)). The proportion of outpatient RSV-positive specimens with another virus detected was higher in the motavizumab compared to the placebo group (76% [31/41] vs. 48% [33/69], respectively (p <0.01)). Influenza type A, HRV-A, parainfluenza viruses and coronaviruses were more likely to be detected in RSV-negative samples than RSV-positive samples (Figures 6.4.A – 6.4.D). No pathogen was more likely to be detected in RSV-positive samples than RSV-negative samples with the exception of *S. pneumoniae* (marginally statistically significant; Figure 6.4.C).

Baseline characteristics associated with subsequent medically-attended wheeze in univariate analyses included family history of asthma, wheeze, hay fever, or eczema, the

presence another child <18 yrs of age in the household, another child <6 yrs of age in the household who attends daycare, and household crowding. Each of these were associated with at least one of the subsequent wheeze outcomes for one of the treatment groups (Table 6.3.A and 6.3.B). All-cause inpatient and outpatient MALRI events occurring during the first 150 days of follow up were associated with all three subsequent medically-attended wheeze outcomes, even after adjusting for baseline risk factors (Supplemental Table 6.1).

We assessed the univariate association of each pathogen detected at inpatient and outpatient MALRI events in the first 150 days of follow up with subsequent medically attended wheeze, and assessed for interactions by treatment group. (Supplemental Tables 6.2 through 6.4). We then did multiple logistic regression analyses including baseline characteristics and pathogen-specific MALRIs that were associated with wheeze in the univariate analyses. The following were associated with one or more subsequent medically attended wheeze outcomes in the adjusted analyses: inpatient MALRI with RSV (motavizumab group only), inpatient or outpatient MALRI with HRV type-A, inpatient MALRI with parainfluenza viruses, outpatient MALRI with HRV type-C, outpatient MALRI with coronaviruses, family history of asthma, and the presence of another child in the household under 6 years of age who attends daycare (Figure 6.5).

Among participants with an RSV hospitalization in the first 150 of follow up, we observed a substantially increased risk of all three subsequent wheeze outcomes in the motavizumab group (i.e. those who broke through motavizumab) compared to the

placebo group (Figure 6.6). Among the 21 participants in the motavizumab arm who developed an RSV inpatient MALRI (i.e. breakthrough disease), there were 31 total inpatient MALRI events [72% (22/31) of which were RSV-associated] during the first 150 days of follow up (Supplemental Table 6.5). Specimens were collected within the analytic window for 97% (30/31) of the events. Testing for additional viruses was completed in 83% (25/30) of the events with a specimen, of which 44% (11/25) were positive for RSV alone, 32% (8/25) were positive for RSV plus at least one other virus, 17% (4/25) were positive only for viruses other than RSV, and 8% (2/25) were negative for all viruses. Samples from the remaining 5 events were not tested because they could not be located. Nine (43%) of the 21 participants with motavizumab breakthrough disease also experienced an outpatient MALRI event during the first 150 days following randomization, of which only one was RSV-associated. Although the majority [81% (17/21)] of participants with motavizumab breakthrough RSV disease had at least one missed or late dose of study drug at some point during the first 150 days of follow up (compared to 633/1,371 [46.2%] of the participants in the motavizumab without breakthrough illness), only 29% (6/21) had a missed or late dose immediately prior to their inpatient RSV-MALRI event (Supplementary Table 6.5). Compared to participants in the motavizumab group who did not have RSV MALRI in the first 150 days of follow up, children with motavizumab breakthrough RSV disease were more likely to experience household crowding, live in a household with other children, including other children who attended daycare, have a family history of wheeze, have a missed or late study dose at some point during the first 150 days of follow up, and to have had an outpatient MALRI (Table 6.4). They also had a greater average number of inpatient

MALRI events in the first 150 days of follow up than placebo participants with at least one RSV-MALRI in the same time period. After adjusting for these variables, as well as the presence of other viruses shown to be associated with increased risk of subsequent wheeze, the interaction by treatment group of the association between inpatient RSV-MALRI and subsequent wheeze outcomes remained statistically significant.

Discussion

As previously reported, a double-blinded randomized trial of the RSV mAb motavizumab found 87% efficacy for the prevention of inpatient RSV MALRI and 71% efficacy for the prevention of outpatient RSV MALRI in a population of healthy, full-term infants who had been randomized to receive either motavizumab or placebo treatment [140]. We evaluated nasopharyngeal specimens from MALRI events that occurred during the first 150 days of follow up in the motavizumab trial for the presence of other pathogens and detected one or more non-RSV viruses in >40% of RSV-MALRI events. We found efficacy for the prevention of MALRI events where RSV was detected alone, as well for as MALRI events where RSV was detected alongside other respiratory viruses. Efficacy for inpatient RSV MALRI events remained consistent regardless of whether RSV was detected alone (87%) or with another virus (86%), suggesting that in lower respiratory illness cases severe enough to merit hospitalization, RSV is likely the etiologic cause of disease. Among outpatient events, however, the efficacy of motavizumab for the prevention of MALRI with RSV detected alone was significantly higher than for MALRI with RSV in combination with other viruses (85% vs. 52%, respectively). This may indicate that RSV is highly likely to be the causal agent for MALRI-RSV events that are

severe (i.e. hospitalized), but that for those of lower severity, treated as outpatients, the finding of RSV in a child is less predictive of causality. To our knowledge, this has not been previously shown in other studies. In some of these cases, co-infecting viruses are likely partially causally associated with illness that would otherwise be uniquely ascribed to RSV on the basis of detecting RSV in a child with a compatible clinical syndrome.

The primary efficacy analysis showed no overall disease replacement occurring with motavizumab prevention of RSV events [140]. In the current analysis, we found evidence for antagonism between RSV and influenza type A, and RSV and human metapneumovirus. Both viruses had higher rates of detection in the motavizumab group at outpatient (but not inpatient) medically attended lower respiratory illness events.

We found a family history of asthma and the presence of a young child in the household who attends day care to be independently associated with subsequent medically attended wheeze. As previously reported, these characteristics were distributed evenly across the treatment groups [282]. We also found parainfluenza, HRV-A, HRV-C, and coronavirus associated MALRI in the first 150 days of follow up to be independently associated with subsequent medically attended wheeze regardless of treatment group. We found that treatment group modified the risk of subsequent medically attended wheeze among those who had RSV MALRI. There was a substantially increased risk of wheezing in the years following RSV prophylaxis among children who were hospitalized with RSV respiratory disease despite motavizumab prophylaxis, compared with children who likewise had had an inpatient RSV MALRI in their first RSV season, but had received placebo. We hypothesize that there are a subset of children who have a host risk factor, or set of risk factors, that not only put them at risk of serious MALRI when they have their first RSV

infection, but that also increase their risk for subsequent wheeze in the future, independent of the RSV infection itself. According to this hypothesis, motavizumab acts like a probe, revealing through prophylaxis failures a subgroup of children in the community who are inherently at high risk for wheezing (Figure 6.7).

Our study differs in important ways from other intervention trials of RSV immunoprophylaxis (palivizumab) where a population-level reduction in subsequent wheezing was observed in the treatment group [175-178]. Only one of these studies was also double-blinded and randomized [175]. In that study, the subsequent wheeze outcome was parental reported wheeze events occurring before the first birthday [175], making it difficult to draw direct comparisons with our subsequent medically attended wheeze outcomes between ages one to three years. Ours was also the only study that included full term rather than preterm infants, and the role of severe RSV illness in subsequent wheezing may be different in this population than in preterm infants.

The increased risk of future wheeze following HRV-lower respiratory illness observed in this study is consistent with several other reports [283]. HRV has been shown to provoke an airway inflammatory response with the induction of respiratory symptoms that can lead to sustained bronchial hyperactivity, predominantly in predisposed individuals with atopic tendencies [284]. We did not have direct measures of atopic characteristics in the infants in our study, but controlling for family history of atopy and asthma did not alter the relationship of HRV-illness to future wheeze. Increased risk of subsequent wheeze following parainfluenza virus and coronavirus illnesses, has not, to our knowledge, been reported in other studies.

Our study was limited by our inability to locate nasopharyngeal specimens from all of the medically attended respiratory events with samples collected, with inpatient samples less likely to be available for testing by the viral multiplex panel if they came from the motavizumab group. We measured rates of viral illnesses in the first RSV season using the full ITT population as the denominator, and only samples with complete testing results could be assigned to the 'RSV only' or 'RSV plus other viruses' numerators in our rate calculations. Because there were more missing results from the motavizumab group, there could have been differential misclassification bias whereby participants in the placebo group were more likely to be assigned as having inpatient RSV or other viral lower respiratory illness compared to the motavizumab group. This could have led to an overestimation of the true efficacy of motavizumab for the prevention of inpatient viral lower respiratory illness. However, our efficacy point estimate for overall prevention of inpatient RSV MALRI was the same as that reported in the parent trial, indicating that the exclusion of events with specimens not tested by the viral multiplex did not bias our efficacy estimates in favor of motavizumab. To assess whether our result of similar efficacy of motavizumab for the prevention of inpatient MALRI with RSV alone versus RSV together with another viruses was influenced by the fact that we did not test three RSV positive samples from the motavizumab group for other viruses, we conducted a sensitivity analysis and found no meaningful change in efficacy even after assuming all three events had other viruses detected. While we did observe increased rates of inpatient MALRI with some non-RSV viruses in the placebo group, the fact that these differences disappeared after excluding those events where RSV was detected along with those viruses suggests that the observed increases were not due to additional availability of

samples from the placebo group – if that had been the case we would expect to see increased detection of non-RSV viruses alone, as well as with RSV, in the placebo group. Additionally, the fact that we did not observe overall efficacy of motavizumab for prevention of MALRI with only non-RSV viruses provides further assurance that misclassification due to missing results did not occur in a substantial number of participants. There was no differential testing of outpatient MALRI events by treatment group, and therefore no obvious source of potential bias in our estimates of efficacy of motavizumab for outpatient MALRI. In analyses of the proportions of MALRIs with specific pathogens detected by treatment group and RSV status, the denominators included only those MALRIs with specimens tested for the pathogen(s) of interest, and missing data should therefore not have affected these results.

Another limitation of this study is that without direct measures of lung function or risk for asthma or atopy in these infants, we were forced to rely family histories as proxy indicators for baseline risk of future disease. Because follow up ended three years after randomization, we were unable to evaluate children at an age where asthma diagnoses could be established. Long-term follow up of the study cohort is now ongoing for asthma outcomes.

The results of this study have implications for future research. The reduced efficacy we observed for outpatient RSV MALRI in combination with other viruses has particular implications for efficacy evaluations of vaccine and monoclonal antibody products that do not produce sterilizing immunity. While a substantial proportion of medically attended RSV disease burden occurs in outpatient settings, the fraction of lower respiratory illness in these settings that is causally associated with RSV is likely reduced compared to

illness severe enough to warrant hospital admission. If the clinical severity threshold for RSV MALRI case definitions in efficacy trials is lowered in an effort to accrue greater numbers of outcome events over a shorter period of time, the product efficacy may be simultaneously driven down as the proportion of those events that are not causally associated with RSV increases. Further work is needed to address the potential impact of RSV prevention on asthma and lung function, which can only be measured later in childhood. The previously reported findings from the motavizumab efficacy trial cast doubt on the assumption that RSV illness prevention will necessarily impact subsequent wheezing and asthma at the population level. Our results indicate the potential for RSV monoclonal antibodies, and possibly vaccines, to impact RSV-associated subsequent wheeze differentially according to the distribution of underlying host risk factors and co-circulating viruses in the population. Further investigation of these host factors is needed, and future trials of RSV vaccines or monoclonal antibodies should be designed to assess long-term respiratory sequelae in their study populations. Finally, our results signal that rates of influenza type A and human metapneumovirus illness could increase when RSV-illness is prevented, presumably due to antagonism between these pathogens. There are suggestions from other studies of potential interference between influenza type A and RSV [285]. This finding should not be over interpreted, however, given that the point estimates for the relative increase in detection of both viruses had confidence intervals that came close to overlapping the null value. It should rather be used to generate hypotheses for future investigations that assess how the prevention of one of these pathogens influences rates of illness with the other.

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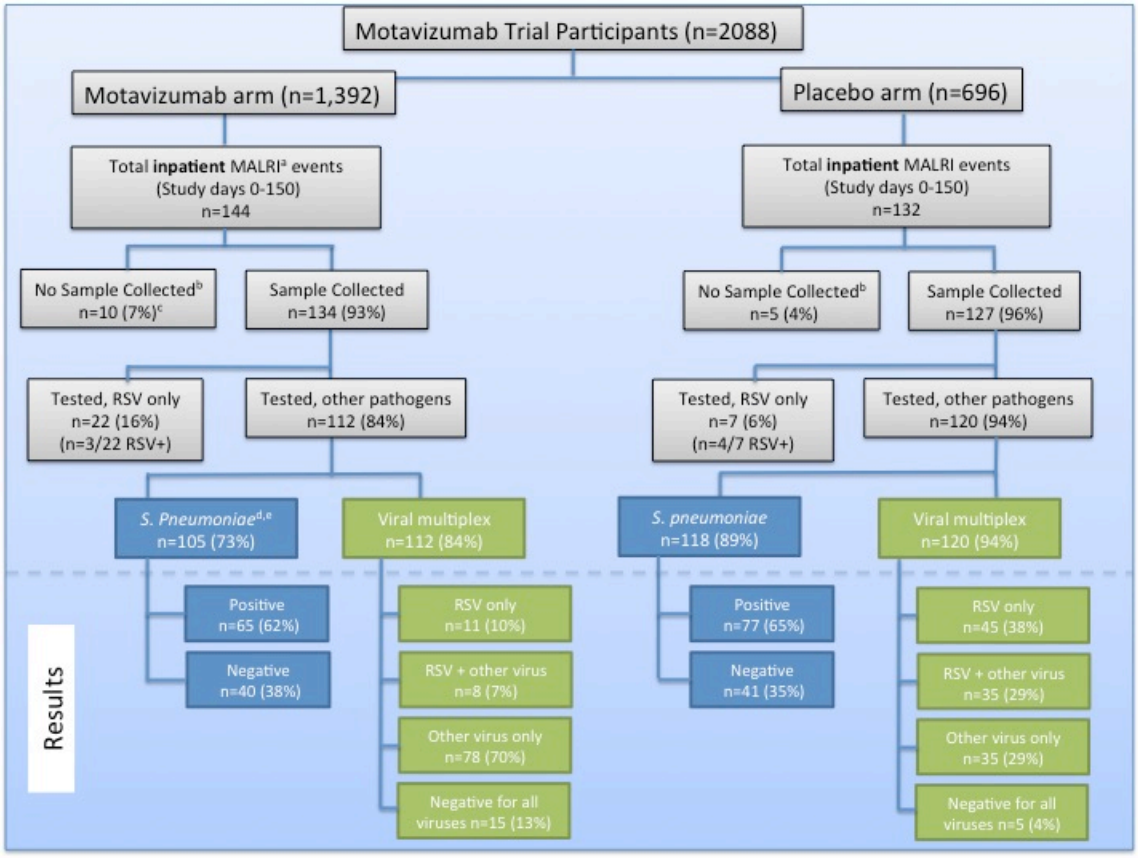
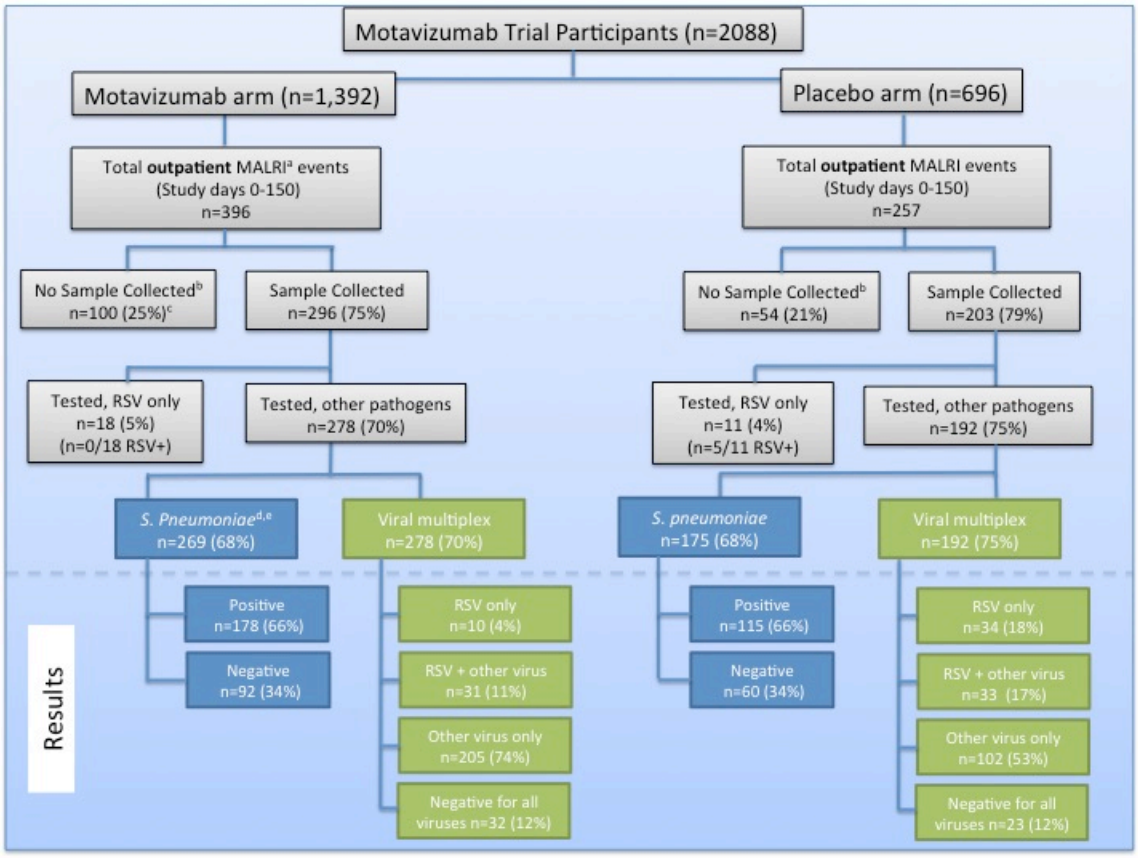
Tables and Figures

Figure 6.1.A Inpatient medically attended lower respiratory illness events during the first 150 days of follow up with samples collected and tested for additional respiratory pathogens



Key:
[a]medically attended lower respiratory illness
[b]within 5 days of MALRI admission date (analytic window)
[C]denominator for sample collection and testing is total number of inpatient MALRI events
[d]*Streptococcus pneumoniae* carriage in the nasopharyx
[e]denominator for results is total number tested
Test result percentages may not sum to 100% due to rounding

Figure 6.1.B Outpatient medically attended lower respiratory illness events during the first 150 days of follow up with samples collected and tested for additional respiratory pathogens



Key:
[a]medically attended lower respiratory illness
[b]within 5 days of MALRI doctor visit (analytic window)
[C]denominator for sample collection and testing is total number of outpatient MALRI events
[d]*Streptococcus pneumoniae* carriage in the nasopharyx
[e]denominator for results is total number tested
Test result percentages may not sum to 100% due to rounding

Table 6.1 Characteristics of MALRI[1] samples according to testing for other viruses, by treatment group and RSV status

	Age		Treatment Group[2]			RSV Status[3]		
	Mean age, mo (SD)	**p-value[1]**	**Mota n (%)**	**Placebo n (%)**	**p-value[4]**	**RSV pos n (%)**	**RSV neg n (%)**	**p-value[2]**
Inpatient Events			**n=144[4]**	**n=132**		**n=105**	**n=156**	
Tested	3.8 (2.2)	0.52	112 (77.8)	120 (90.9)	**<0.01**	99 (94.3)	133 (85.3)	**0.02**
Not Tested	4.1 (2.4)		32 (22.2)	12 (9.1)		6 (5.7)	23 (14.7)	
Outpatient Events			**n=396**	**n=257**		**n=112**	**n=387**	
Tested	5.3 (2.1)	0.40	280 (70.7)	193 (75.1)	0.22	107 (95.5)	363 (93.8)	0.48
Not Tested	5.0 (2.4)		116 (29.3)	64 (24.9)		5 (7.0)	24 (6.2)	
All Events			**n=540**	**n=389**		**n=217**	**n=543**	
Tested	4.8 (2.3)	0.67	392 (72.6)	313 (80.5)	**<0.01**	206 (93.6)	496 (85.2)	0.09
Not Tested	4.7 (2.4)		148 (27.4)	76 (19.5)		11 (6.4)	47 (14.8)	

[1]Medically attended lower respiratory illness
[2]Denominator for testing by treatment group includes all MALRI events regardless of whether a sample was collected within the analytic window
[3]Denominator for testing by RSV status is the subset of events with sample collected and tested for RSV
[4] Fisher's exact test

Figure 6.2 Relative risk of medically attended lower respiratory illness outcomes in the first 150 days of follow up in the motavizumab treatment group compared to the placebo treatment group

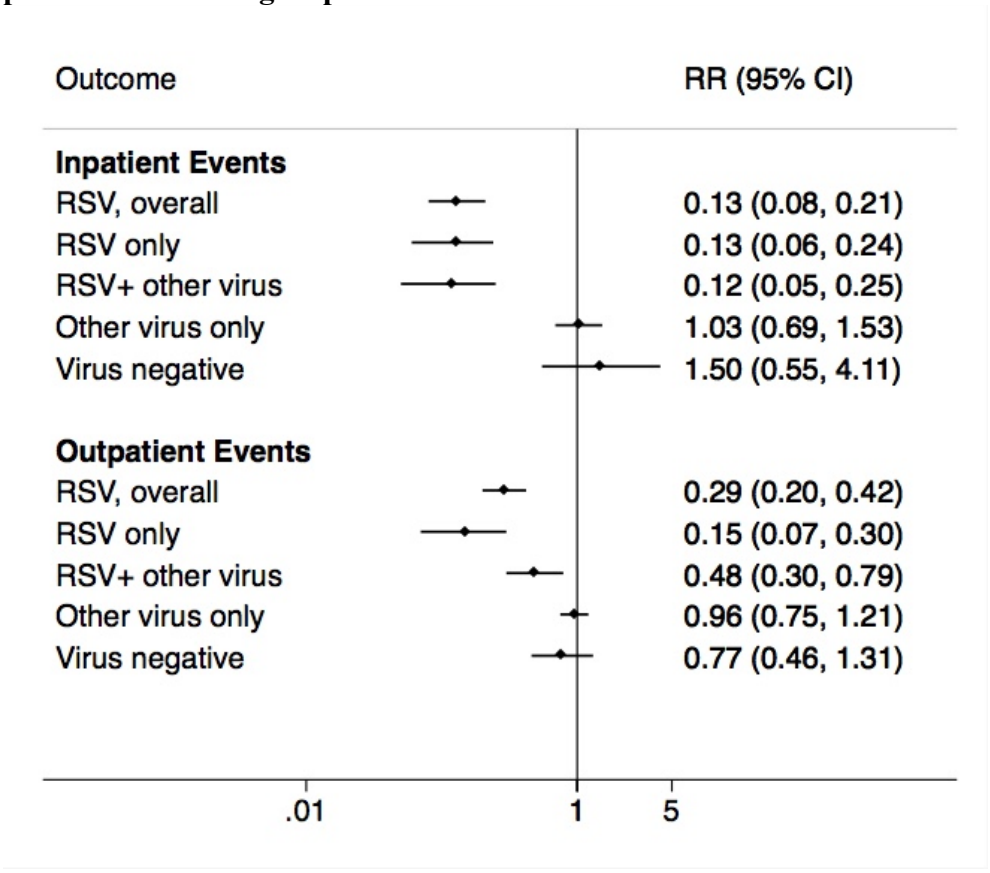


Table 6.2.A Rates of inpatient medically attended lower respiratory illness in the first 150 days of follow up, by pathogen and treatment group

	Motavizumab (n=1,392) N (%)	Placebo (n=696) N (%)	Relative risk (95% CI)	p-value[1]	Absolute rate reduction, cases per 100 children (95%CI)
RSV only[2]	11 (0.8)	44 (6.3)	**0.13 (0.06, 0.24)**	**<0.001**	**5.5 (3.7, 7.4)**
RSV with other virus	8 (0.6)	34 (4.9)	**0.12 (0.5, 0.25)**	**<0.001**	**4.3 (2.7, 6.0)**
Other virus only	70 (5.0)	34 (4.9)	1.03 (0.69, 1.53)	0.91	-0.1 (-2.1, 1.8)
Virus Negative	15 (1.1)	5 (0.7)	1.50 (0.55, 4.11)	0.49	-0.4 (-1.2, 0.5)
RSV with 1 other virus	5 (0.4)	25 (3.6)	**0.10 (0.04, 0.26)**	**<0.01**	**3.2 (1.8, 4.7)**
RSV with >1 other virus	3 (0.2)	9[3] (1.3)	**0.17 (0.05, 0.61)**	**<0.01**	**1.1 (0.2, 2.0)**
Other virus only					
1 virus	48 (3.5)	19 (2.7)	1.26 (0.75, 2.13)	0.43	-0.7 (-2.2, 0.8)
>1 virus	25 (1.8)	16[3] (2.3)	0.78 (0.42, 1.45)	0.50	0.5 (-0.8, 1.8)
Rhinovirus[4] (any)	32 (2.3)	32 (4.6)	**0.50 (0.31, 0.81)**	**<0.01**	**2.3 (0.6, 4.0)**
Rhinovirus with RSV	2 (0.1)	18 (2.6)	**0.06 (0.01, 0.24)**	**<0.001**	**2.4 (1.2, 3.6)**
Rhinovirus without RSV	31 (2.2)	16 (2.3)	0.97 (0.53, 1.760	1.00	0.0 (-1.3, 1.4)
Rhinovirus type A	18 (1.3)	14 (2.0)	0.64 (0.32, 1.28)	0.26	0.7 (0.00, 1.9)
Rhinovirus type A with RSV	0 (0.0)	6 (0.9)	**-**	**<0.01**	**0.9 (0.2, 1.5)**
Rhinovirus type A without RSV	18 (1.3)	9 (1.3)	1.00 (0.45, 2.21)	1.00	0.0 (-1.0, 1.0)
Rhinovirus type B	2 (0.1)	2 (0.3)	0.50 (0.07, 3.54)	0.61	0.1 (-0.3, 0.6)
Rhinovirus type B with RSV	0 (0.0)	1 (0.1)	-	0.33	0.1 (-0.01, 0.4)
Rhinovirus type B without RSV	2 (0.0)	1 (0.1)	1.00 (0.06, 11.01)	1.00	0.0 (-0.3, 0.3)
Rhinovirus type C	15 (1.1)	19 (2.7)	**0.39 (0.21, 0.77)**	**<0.01**	**1.7 (0.3, 3.0)**
Rhinovirus type C with RSV	2 (0.1)	11 (1.6)	**0.09 (0.02, 0.41)**	**<0.001**	**1.4 (0.5, 2.4)**
Rhinovirus type C without RSV	14 (1.0)	8 (1.2)	0.88 (0.37, 2.08)	0.82	0.1 (-0.8, 1.1)
Parainfluenza Virus 1-4	14 (1.0)	16 (2.3)	**0.44 (0.21, 0.89)**	**0.03**	**1.3 (0.0, 2.5)**
Parainfluenza Virus 1-4 with RSV	1 (0.1)	8 (1.2)	**0.06 (0.01, 0.50)**	**<0.01**	**1.1 (0.3, 1.9)**

	Motavizumab (n=1,392) N (%)	Placebo (n=696) N (%)	Relative risk (95% CI)	p-value[1]	Absolute rate reduction, cases per 100 children (95%CI)
Parainfluenza Virus 1-4 without RSV	13 (0.9)	10 (1.4)	0.65 (0.29, 1.47)	0.37	0.5 (-0.5, 1.5)
Human metapneumovirus	12 (0.9)	12 (1.7)	0.50 (0.23, 1.11)	0.09	0.9 (-0.2, 1.2)
Human metapneumovirus with RSV	1 (0.1)	5 (0.7)	**0.10 (0.01, 0.85)**	**0.02**	**0.6 (0.0, 1.3)**
Human metapneumovirus without RSV	11 (0.8)	7 (1.0)	0.79 (0.31, 2.02)	0.62	0.2 (-0.7, 1.1)
Adenovirus	18 (1.3)	10 (1.4)	0.90 (0.42, 1.94)	0.84	0.1 (-0.1, 1.2)
Adenovirus with RSV	4 (0.3)	6 (0.9)	0.33 (0.09, 1.18)	0.10	0.6 (-0.1, 1.3)
Adenovirus without RSV	15 (1.1)	4 (0.6)	1.88 (0.62, 5.62)	0.33	0.5 (-1.3, 0.3)
Influenza type A	12 (0.9)	7 (1.0)	0.86 (0.34, 2.17)	0.81	0.1 (-0.7, 1.0)
Influenza type A with RSV	1 (0.1)	1 (0.1)	0.50 (0.03, 7.98)	1.00	0.0 (-0.2, 0.3)
Influenza type A without RSV	11 (0.8)	6 (0.9)	0.92 (0.34, 2.47)	1.00	0.0 (-0.8, 0.9)
Influenza type B	5 (0.4)	1 (0.1)	2.50 (0.29, 21.36)	0.67	-0.2 (-0.6, 0.2)
Influenza type B with RSV	0 (0.0)	0 (0.0)	-	-	-
Influenza type B without RSV	5 (0.4)	1 (0.1)	2.50 (0.29, 21.36)	0.67	-0.2 (-0.6, 0.2)
Enterovirus	1 (0.1)	1 (0.1)	0.50 (0.03, 7.98)	1.00	0.0 (-0.2, 0.4)
Enterovirus with RSV	0 (0.0)	0 (0.0)	-	-	-
Enterovirus without RSV	1 (0.1)	1 (0.1)	0.50 (0.03, 7.98)	1.00	0.0 (-0.2, 0.4)
Coronavirus[5]	13 (0.9)	15 (2.2)	**0.43 (0.21, 0.91)**	**0.03**	**1.2 (0.0, 2.4)**
Coronavirus with RSV	1 (0.1)	5 (0.7)	**0.10 (0.01, 0.85)**	**0.02**	**0.6 (0.0, 1.3)**
Coronavirus without RSV	12 (0.9)	10 (1.4)	0.60 (0.26, 1.38)	0.26	0.6 (-0.4, 1.6)
Bocavirus	4 (0.3)	2 (0.3)	1.00 (0.18, 5.45)	1.00	0.0 (-0.5, 0.5)
Bocavirus with RSV	1 (0.1)	2 (0.3)	0.25 (0.02, 2.75)	0.26	0.2 (-0.2, 0.6)
Bocavirus without RSV	3 (0.2)	0 (0.0)	-	0.56	-0.2 (-0.5, 0.0)

[1]Fishers exact test, alpha =0.05
[2]Excludes n=2 motavizumab and n=2 placebo participants positive for RSV in the motavizumab trial but without a specimen available for testing for additional viruses. Only samples tested for RSV and other viruses could be categorized as 'RSV only' or 'RSV with other viruses'. Denominators for calculation of relative risk and absolute rate reduction include the entire intention to treat population for each treatment arm.
[3]One placebo participant had both a non-RSV viral event with 1 virus only, and with multiple viruses
[4]Some Rhinovirus positive secretions were positive for more than one subtype, in which case they were counted as both; therefore the sum of participants with HRV-A + HRV-B + HRV-C > total participants HRV.
[5]Includes coronavirus types 229E, NL63, OC43, and HKU1

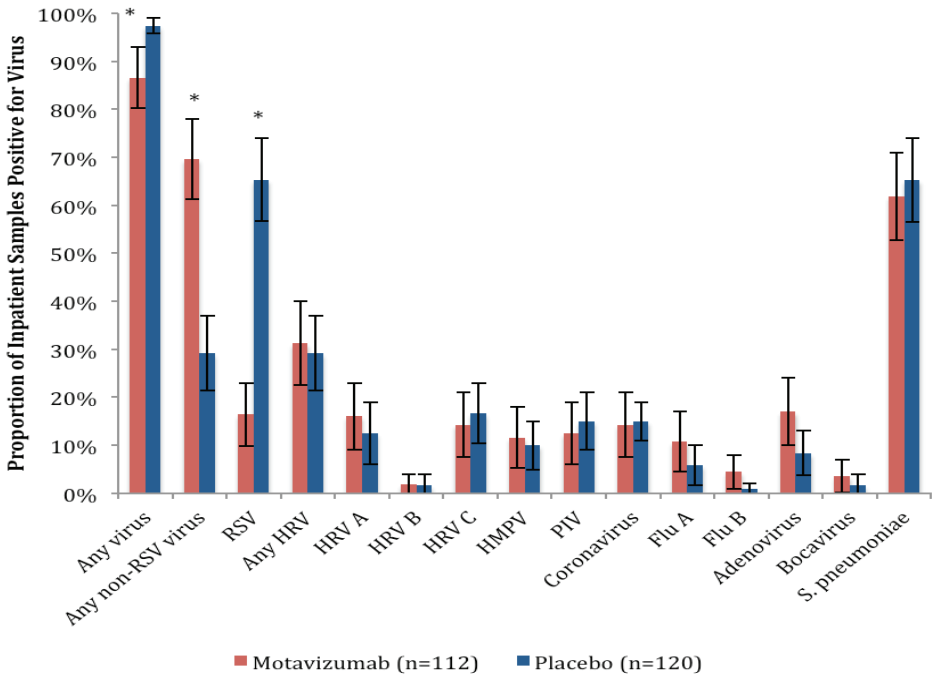
Table 6.2.B Rates of outpatient medically attended lower respiratory illness in the first 150 days of follow up, by pathogen and treatment group

	Motavizumab (n=1,392) N (%)	Placebo (n=696) N (%)	Relative risk (95% CI)	p-value[1]	Absolute rate reduction, cases per 100 children (95%CI)
RSV only[2]	10 (0.7)	34 (4.9)	**0.15 (0.07, 0.30)**	**<0.001**	**4.2 (2.5, 5.8)**
RSV with other virus	30 (2.2)	31 (4.5)	**0.48 (0.30, 0.79)**	**<0.01**	**2.3 (0.6, 4.0)**
Other virus only	172 (12.4)	90 (12.9)	0.96 (0.75, 1.21)	0.73	0.6 (-2.5, 3.6)
Virus Negative	34 (2.4)	22 (3.2)	0.77 (0.46, 1.31)	0.39	0.7 (-0.8, 2.3)
RSV with 1 other virus	18 (1.3)	25 (3.6)	**0.36 (0.20, 0.66)**	**<0.01**	**2.3 (0.8, 3.8)**
RSV with >1 other virus	13 (0.9)	8 (1.2)	0.81 (0.34, 1.95)	0.65	0.2 (-0.7, 1.2)
Other virus only					
1 virus	121 (8.7)	73 (10.5)	0.83 (0.63, 1.09)	0.20	1.8 (-0.9, 4.5)
>1 virus	62 (4.5)	19 (2.7)	1.63 (0.98, 2.71)	0.06	-1.7 (-3.3, -0.1)
Rhinovirus[3] (any)	109 (7.8)	79 (11.4)	**0.69 (0.52, 0.91)**	**<0.01**	**3.5 (0.8, 6.3)**
Rhinovirus with RSV	17 (1.2)	17 (2.4)	**0.50 (0.26, 0.97)**	**0.04**	**1.2 (-0.1, 2.5)**
Rhinovirus without RSV	96 (6.9)	65 (9.3)	0.74 (0.55, 1.00)	0.05	2.4 (-0.1, 5.0)
Rhinovirus type A	57 (4.1)	45 (6.5)	**0.63 (0.43, 0.93)**	**0.03**	**2.4 (0.3, 4.5)**
Rhinovirus type A with RSV	9 (0.7)	9 (1.3)	0.50 (0.20, 1.25)	0.26	0.6 (-0.3, 1.6)
Rhinovirus type A without RSV	48 (3.5)	36 (5.2)	0.67 (0.44, 1.02)	0.08	1.7 (-0.2, 3.6)
Rhinovirus type B	6 (0.4)	2 (0.3)	1.50 (0.30, 7.41)	0.73	0.1 (-0.7, 0.41)
Rhinovirus type B with RSV	1 (0.07)	0 (0.0)	-	1.00	-0.1 (-0.2, 0.1)
Rhinovirus type B without RSV	5 (0.4)	2 (0.3)	1.25 (0.24, 6.43)	1.00	-0.1 (-0.6, 0.4)
Rhinovirus type C	48 (3.5)	37 (5.3)	**0.65 (0.43, 0.99)**	**0.04**	1.9 (-0.1, 3.8)
Rhinovirus type C with RSV	6 (0.4)	9 (1.3)	0.33 (0.12, 0.93)	0.05	0.9 (0.0, 1.8)
Rhinovirus type C without RSV	44 (3.2)	29 (4.2)	0.76 (0.48, 1.20)	0.26	1.0 (-0.7, 2.8)
Parainfluenza Virus 1-4	40 (2.9)	15 (2.2)	1.33 (0.74, 2.40)	0.39	-0.7 (-2.1, 0.7)
Parainfluenza Virus 1-4 with RSV	1 (0.1)	1 (0.1)	0.50 (0.03, 7.98)	1.00	0.1 (-0.2, 0.4)

	Motavizumab (n=1,392) N (%)	Placebo (n=696) N (%)	Relative risk (95% CI)	p-value[1]	Absolute rate reduction, cases per 100 children (95%CI)
Parainfluenza Virus 1-4 without RSV	39 (2.8)	14 (2.0)	1.39 (0.76, 2.55)	0.31	-0.8 (-2.1, 0.6)
Human metapneumovirus	34 (2.4)	11 (1.6)	1.55 (0.79, 3.03)	0.26	-0.8 (-2.1, 0.4)
Human metapneumovirus with RSV	5 (0.4)	6 (0.9)	0.42 (0.13, 1.36)	0.20	0.5 (-0.3, 1.3)
Human metapneumovirus without RSV	29 (2.1)	5 (0.7)	**2.90 (1.13, 7.46)**	**0.03**	**-1.4 (-2.3, -0.4)**
Adenovirus	27 (1.9)	15 (2.1)	0.90 (0.48, 1.68)	0.74	0.2 (-1.1, 1.5)
Adenovirus with RSV	5 (0.4)	8 (1.2)	**0.31 (0.10, 0.95)**	**0.04**	**0.8 (-0.1, 1.6)**
Adenovirus without RSV	23 (1.7)	8 (1.2)	1.44 (0.65, 3.20)	0.45	-0.5 (-1.5, 0.5)
Influenza type A	24 (1.7)	5 (0.7)	2.40 (0.92, 6.26)	0.06	-1.0 (-1.9, -0.1)
Influenza type A with RSV	1 (0.1)	1 (0.1)	0.50 (0.03, 7.98)	1.00	0.1 (-0.2, 0.4)
Influenza type A without RSV	23 (1.7)	4 (0.6)	**2.88 (0.99, 8.28)**	**0.04**	**-1.1 (-0.2, -2.0)**
Influenza type B	9 (0.7)	5 (0.7)	0.90 (0.30, 2.68)	1.00	0.1 (-0.7, 0.8)
Influenza type B with RSV	2 (0.1)	0 (0.0)	-	0.55	-0.1 (-0.3, 0.1)
Influenza type B without RSV	7 (0.5)	5 (0.7)	0.70 (0.22, 2.20)	0.55	0.2 (-0.5, 0.9)
Enterovirus	3 (0.2)	0 (0.0)	-	0.55	-0.2 (0.5, 0.0)
Enterovirus with RSV	1 (0.1)	0 (0.0)	-	1.00	-0.1 (-0.2, 0.1)
Enterovirus without RSV	2 (0.1)	0 (0.0)	-	0.55	-0.1 (-0.3, 0.1)
Coronavirus[4]	47 (3.4)	13 (1.9)	1.81 (0.98, 3.32)	0.05	-1.5 (-2.9, -1.3)
Coronavirus with RSV	8 (0.6)	3 (0.4)	1.33 (0.35, 5.01)	0.76	-0.1 (-0.8, 0.5)
Coronavirus without RSV	39 (2.8)	10 (1.4)	1.95 (0.98, 3.88)	0.07	-1.4 (-2.6, -1.3)
Bocavirus	26 (1.2)	9 (1.3)	0.89 (0.39, 2.00)	0.83	0.1 (-0.9, 1.2)
Bocavirus with RSV	4 (0.3)	6 (0.9)	0.33 (0.09, 1.78)	0.09	0.6 (-0.2, 1.3)
Bocavirus without RSV	12 (0.9)	3 (0.4)	2.00 (0.57, 7.06)	0.41	-0.4 (-1.1, 0.3)

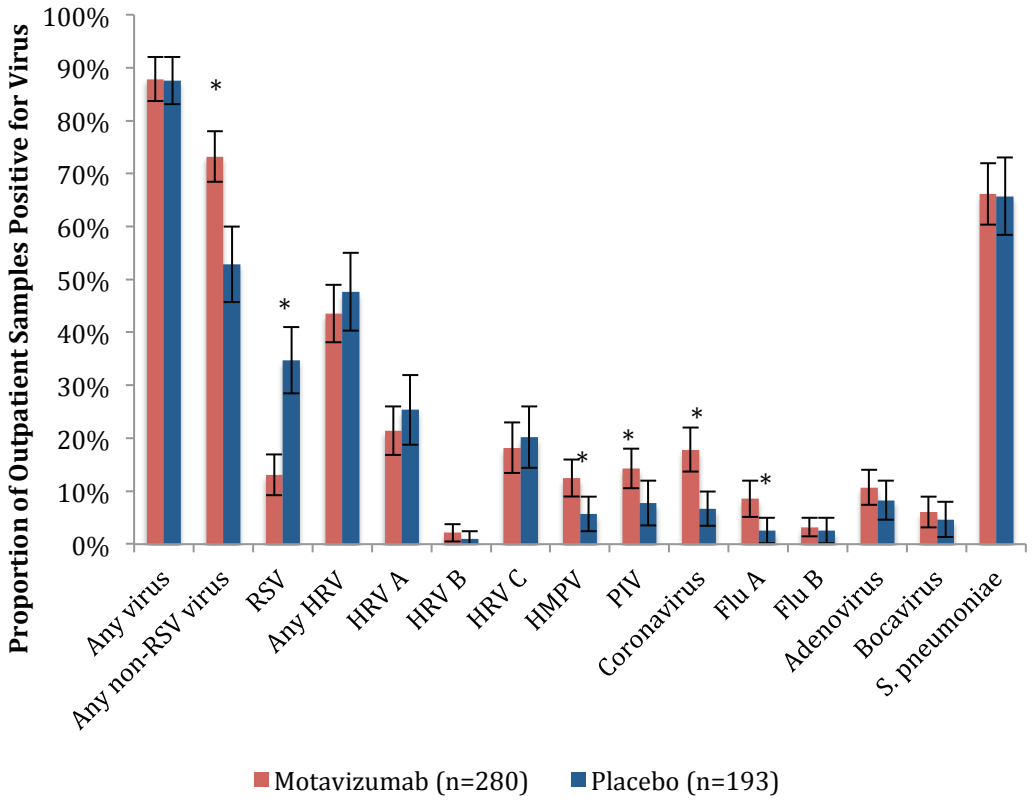
[1]Fishers exact test, alpha =0.05
[2]Excludes n=2 placebo participants positive for RSV in the motavizumab trial but without a specimen available for testing for additional viruses. Only samples tested for RSV and other viruses could be categorized as 'RSV only' or 'RSV with other viruses'. Denominators for calculation of relative risk and absolute rate reduction include the entire intention to treat population for each treatment arm.
[3]Some Rhinovirus positive secretions were positive for more than one subtype, in which case they were counted as both; therefore the sum of participants with HRV-A + HRV-B + HRV-C > total participants HRV.
[4]Includes coronavirus types 229E, NL63, OC43, and HKU1

Figure 6.3.A Proportion of inpatient medically attended lower respiratory illness events in first 150 days of follow up with pathogen detected, with 95% confidence intervals



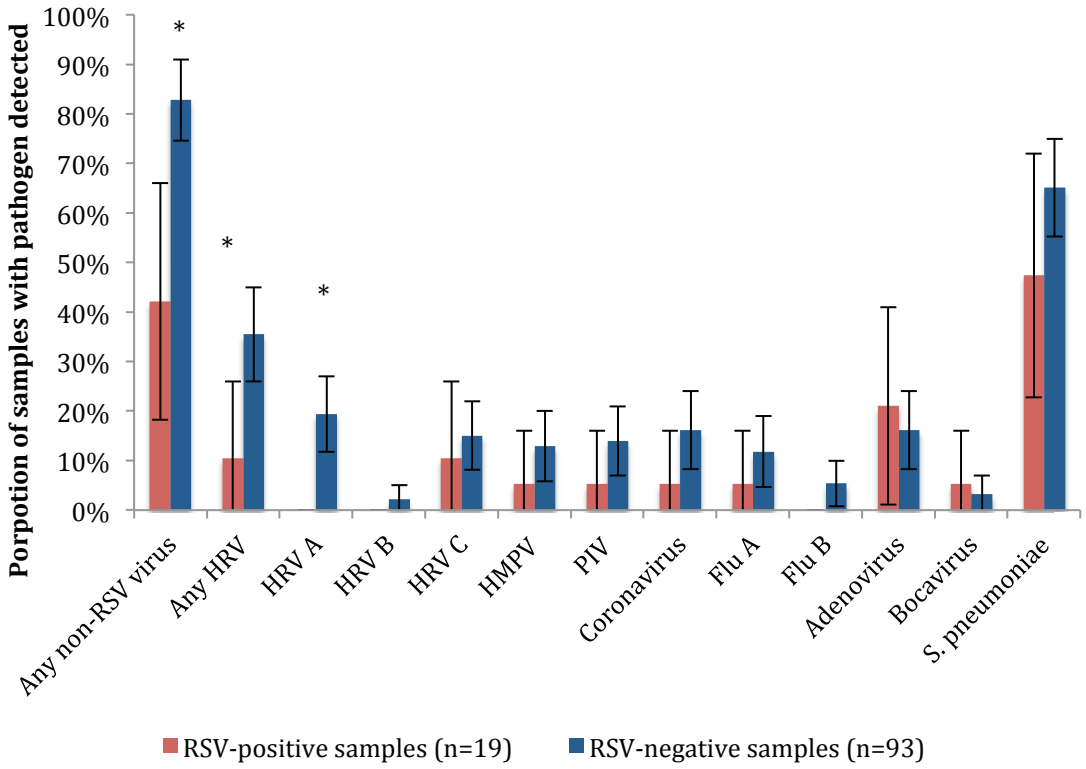
Denominators include only samples tested for the pathogen in question (Motavizumab group: n=134 for RSV testing; n=112 for other viruses; n=105 for *S. pneumoniae*. Placebo group: n=127 for RSV testing; n=120 for other viruses; n=118 for *S. pneumoniae*.)
Any HRV: any of human rhinovirus subtypes A, B, or C; HMPV: human metapneumovirus; PIV: any of parainfluzenza viruses 1-4; Flu A: influenza type A; Flu B: influenza type B; S. pneumoniae: *Streptococcus pneumoniae* carriage in the nasopharynx.
*p<0.0

Figure 6.3.B Proportion of outpatient medically attended lower respiratory illness events in first 150 days of follow up with pathogen detected, with 95% confidence intervals



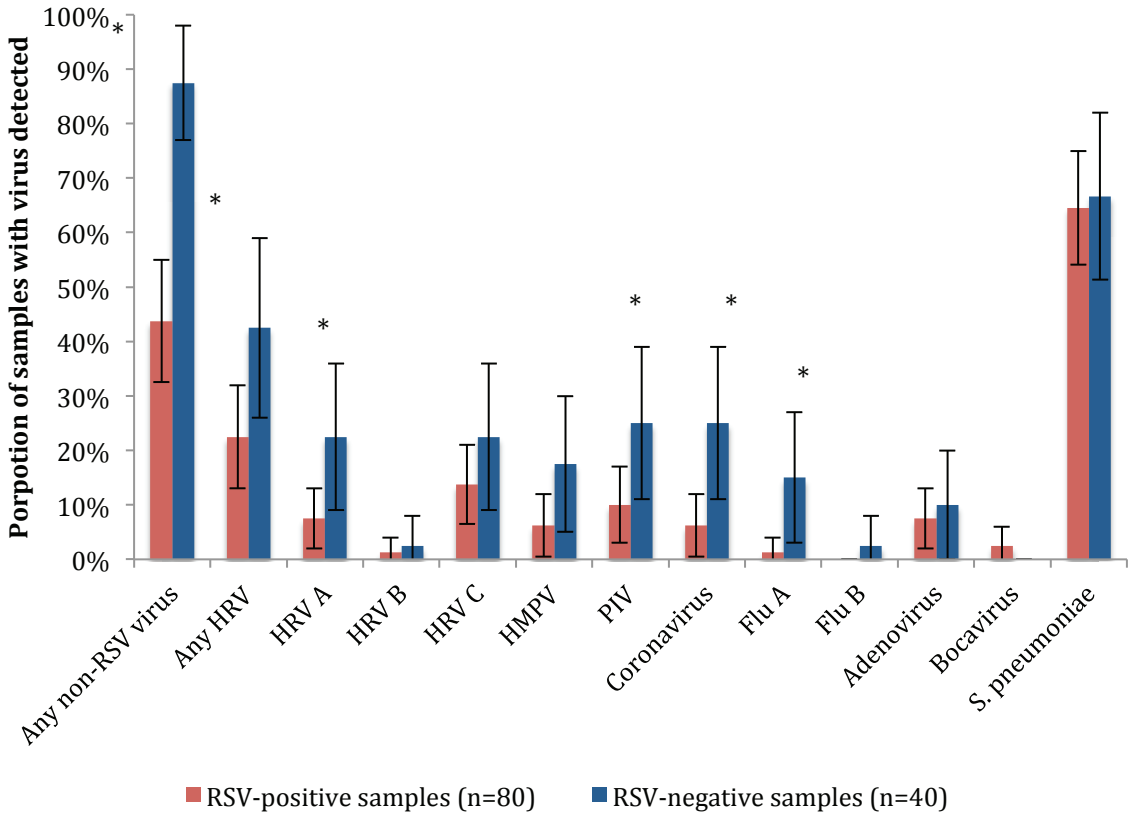
Denominators include only samples tested for the pathogen in question (Motavizumab group: n=320 for RSV testing; n=280 for other viruses; n=269 for *S. pneumoniae*. Placebo group: n=213 for RSV testing; n=193 for other viruses; n=175 for *S. pneumoniae*.)
Any HRV: any of human rhinovirus subtypes A, B, or C; HMPV: human metapneumovirus; PIV: any of parainfluzenza viruses 1-4; Flu A: influenza type A; Flu B: influenza type B; S. pneumoniae: *Streptococcus pneumoniae* carriage in the nasopharynx.
*$p<0.05$

Figure 6.4.A Detection of pathogens at inpatient medically attended respiratory illness events occurring in the first 150 days of follow up in the motavizumab group by RSV- status, with 95% confidence intervals



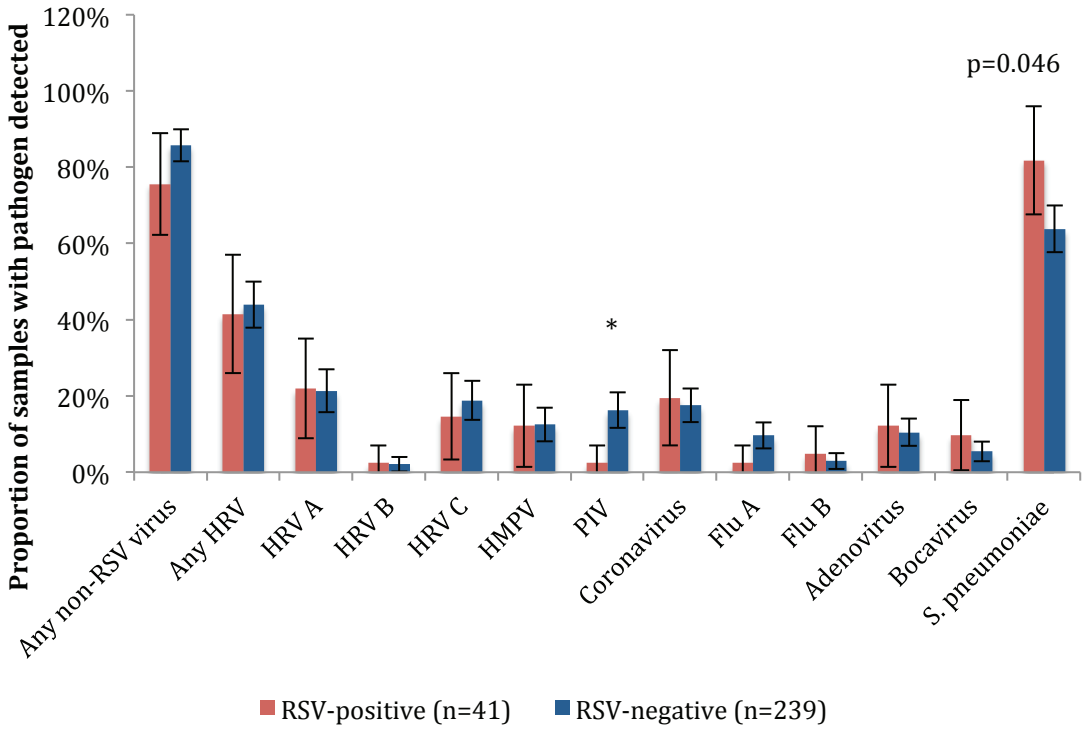
Denominators include only samples tested for the pathogen in question (RSV positive samples: n=19 for other viruses; n=19 for *S. pneumoniae*. RSV negative samples: n=93 for other viruses; n=86 for *S. pneumoniae*).
Any HRV: any of human rhinovirus subtypes A, B, or C; HMPV: human metapneumovirus; PIV: any of parainfluenza viruses 1-4; Flu A: influenza type A; Flu B: influenza type B; S. pneumoniae: *Streptococcus pneumoniae* carriage in the nasopharynx.
*p<0.05

Figure 6.4.B Detection of pathogens at inpatient medically attended respiratory illness events occurring in the first 150 days of follow up in the placebo group by RSV- status, with 95% confidence intervals



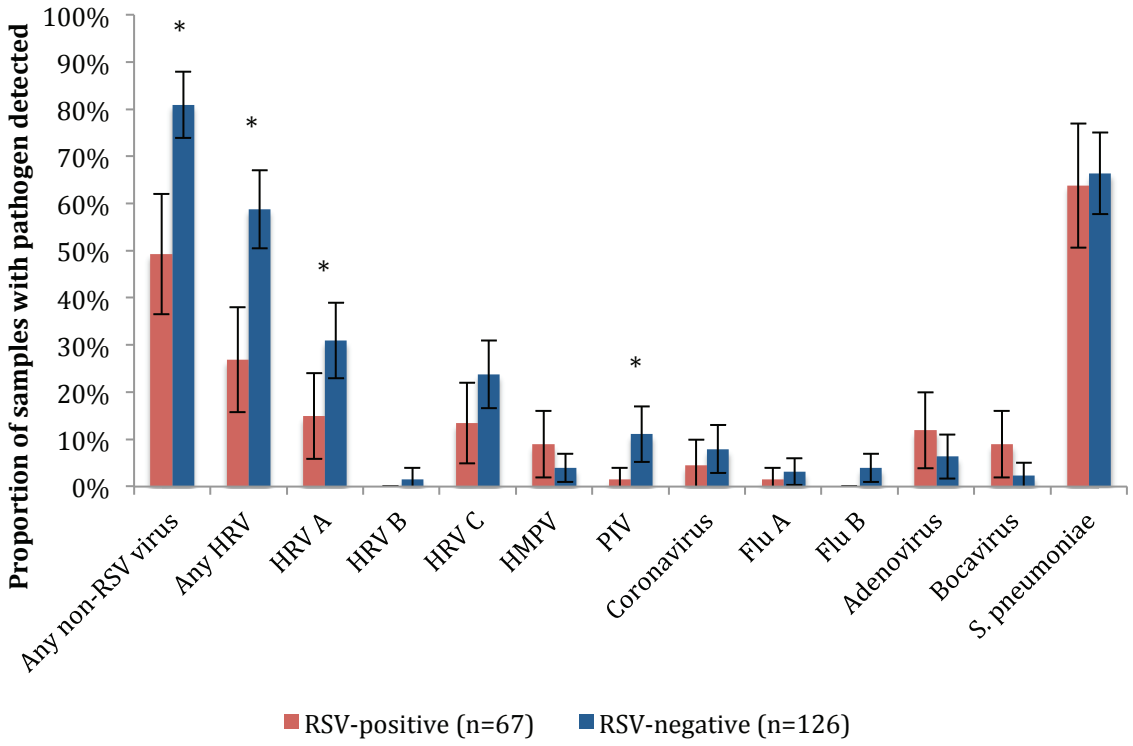
Denominators include only samples tested for the pathogen in question (RSV positive samples: n=80 for other viruses; n=79 for *S. pneumoniae*. RSV negative samples: n=40 for other viruses; n=39 for *S. pneumoniae*).
Any HRV: any of human rhinovirus subtypes A, B, or C; HMPV: human metapneumovirus; PIV: any of parainfluenza viruses 1-4; Flu A: influenza type A; Flu B: influenza type B; S. pneumoniae: *Streptococcus pneumoniae* carriage in the nasopharynx.
*p<0.05

Figure 6.4.C Detection of pathogens at outpatient medically attended respiratory illness events occurring in the first 150 days of follow up in the motavizumab group by RSV- status, with 95% confidence intervals



Denominators include only samples tested for the pathogen in question (RSV positive samples: n=41 for other viruses; n=33 for *S. pneumoniae*. RSV negative samples: n=239 for other viruses; n=235 for *S. pneumoniae*).
Any HRV: any of human rhinovirus subtypes A, B, or C; HMPV: human metapneumovirus; PIV: any of parainfluenza viruses 1-4; Flu A: influenza type A; Flu B: influenza type B; S. pneumoniae: *Streptococcus pneumoniae* carriage in the nasopharynx.
*p<0.05

Figure 6.4.D Detection of pathogens at outpatient medically attended respiratory illness events occurring in the first 150 days of follow up in the placebo group by RSV- status, with 95% confidence intervals



Denominators include only samples tested for the pathogen in question (RSV positive samples: n=67 for other viruses; n=58 for *S. pneumoniae*. RSV negative samples: n=126 for other viruses; n=116 for *S. pneumoniae*).
Any HRV: any of human rhinovirus subtypes A, B, or C; HMPV: human metapneumovirus; PIV: any of parainfluenza viruses 1-4; Flu A: influenza type A; Flu B: influenza type B; S. pneumoniae: *Streptococcus pneumoniae* carriage in the nasopharynx.
*p<0.05

Table 6.3.A Baseline risk factors for subsequent medically attended wheeze outcomes in the motavizumab group

	≥1 medically attended wheeze event N (%)	RR (95%CI)	Serious early childhood wheeze N (%)	RR (95%CI)	Recurrent wheeze N (%)	RR (95%CI)
Male						
Yes (n=696)	157 (22.6)	0.02 (0.76, 1.11)	81 (11.6)	0.81 (0.62, 1.06)	14 (2.0)	0.7 (0.36, 1.37)
No (n=696)	171 (24.6)		100 (14.4)		20 (2.9)	
Attends day care						
Yes (n=43)	11 (25.6)	1.09 (0.65, 1.83)	9 (20.9)	1.64 (0.90, 2.98)	3 (7.0)	3.03 (0.96, 9.54)
No (n=1348)	317 (23.5)		172 (12.8)		31 (23.0)	
Family history of asthma[1]						
Yes (n=292)	100 (34.2)	**1.65 (1.36, 2.01)**	56 (19.2)	**1.69 (1.26, 2.25)**	15 (5.1)	**2.97 (1.53, 5.78)**
No (n=1099)	228 (20.7)		125 (11.4)		19 (0.2)	
Family history of wheeze						
Yes (n= 200)	66 (33.0)	**1.50 (1.20, 1.88)**	35 (17.5)	**1.42 (1.02, 2.00)**	6 (3.0)	1.28 (0.54, 3.05)
No (n= 1192)	262 (22.0)		146 (12.2)		28 (2.3)	
Family history of hay fever						
Yes (n= 139)	43 (30.1)	**1.36 (1.04, 1.78)**	24 (17.3)	1.38 (0.93, 2.04)	6 (4.3)	1.93 (0.81, 4.58)
No (n= 1251)	285 (22.8)		157 (12.5)		28 (2.2)	
Family history of eczema						
Yes (n=134)	39 (29.1)	1.26 (0.95, 1.68)	19 (14.2)	1.10 (0.71, 1.71)	5 (3.7)	1.62 (0.64, 4.11)
No (n=1256)	289 (23.0)		162 (12.9)		29 (2.3)	
Smoke exposure						
Yes (n= 957)	221 (23.1)	0.94 (0.77, 1.15)	122 (12.7)	0.94 (0.70, 1.25)	24 (2.5)	1.09 (0.53, 2.26)
No (n=434)	107 (24.7)		59 (13.6)		10 (2.3)	
Household crowding						
Yes (n=1088)	254 (23.3)	0.96 (0.76, 1.20)	130 (11.9)	0.71 (0.53, 0.96)	28 (2.6)	1.30 (0.54, 3.11)
No (n=303)	74 (24.4)		51 (16.8)		6 (2.0)	
Other child <18 yrs in household						
Yes (n=1212)	288 (23.8)	0.69 (0.49, 0.97)	31 (2.6)	**0.69 (0.49, 0.97)**	31 (2.6)	1.53 (0.47, 4.94)
No (n=179)	40 (22.3)		3 (1.7)		3 (1.7)	
Other child <6 yrs in household						
Yes (n=899)	221 (24.6)	1.13 (0.92, 1.39)	115 (12.8)	0.96 (0.72, 1.27)	20 (2.2)	0.78 (0.40, 1.54)
No (n=493)	107 (21.7)		66 (13.4)		14 (2.8)	
Other child <6 yrs in household, in day care						
Yes (n=144)	41 (28.5)	1.24 (0.94, 1.64)	28 (19.4)	**1.59 (1.10, 2.28)**	8 (5.6)	**2.67 (1.23, 5.78)**
No (n=1248)	287 (23.0)		153 (12.2)		26 (2.1)	

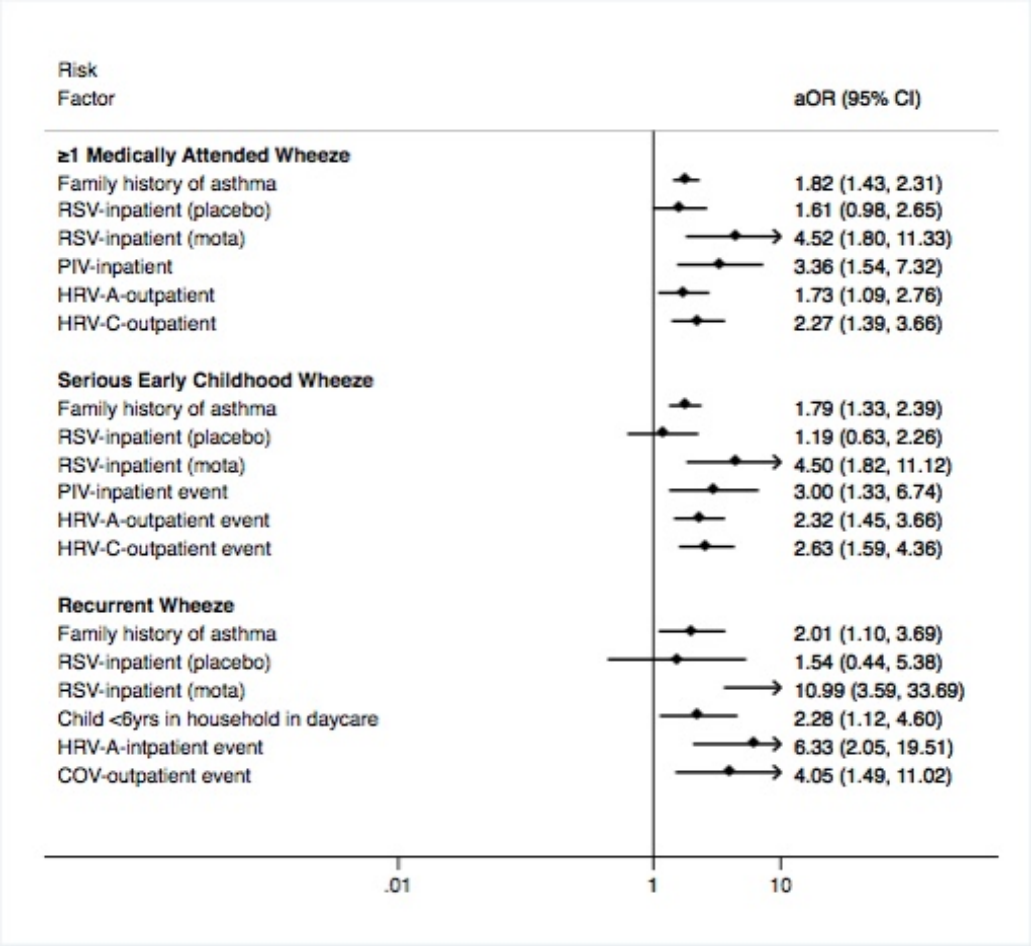
[1]Family history of asthma, wheeze, eczema or hay fever defined as mother, father or sibling having a history of asthma, wheeze, eczema or hay fever.

Table 6.3.B Baseline risk factors for subsequent medically attended wheeze outcomes in the placebo group

[a]Family history of asthma, wheeze, eczema or hay fever defined as mother, father or sibling having a history of asthma, wheeze, eczema or hay fever.

	≥1 medically attended wheeze event N (%)	RR (95%CI)	Serious early childhood wheeze N (%)	RR (95%CI)	Recurrent wheeze N (%)	RR (95%CI)
Male						
Yes (n=360)	83 (24.7)	0.99 (0.76, 1.28)	45 (13.3)	1.21 (0.81, 1.80)	8 (2.4)	1.07 (0.41, 2.82)
No (n=336)	90 (25.0)		40 (11.1)		8 (2.2)	
Attends day care						
Yes (n=21)	2 (9.5)	0.38 (0.09, 1.41)	1 (4.8)	0.38 (0.06, 2.62)	0 (0.0)	-
No (n=675)	171 (25.3)		84 (12.4)		16 (2.4)	
Family history of asthma[a]						
Yes (n= 135)	46 (34.1)	**1.50 (1.14, 1.99)**	26 (19.3)	**1.83 (1.20, 2.79)**	3 (2.2)	0.96 (0.28, 3.31)
No (n=560)	127 (22.7)		59 (10.5)		13 (2.3)	
Family history of wheeze						
Yes (n= 103)	38 (36.9)	**1.62 (1.21, 2.17)**	23 (22.3)	**2.14 (1.39, 3.28)**	3 (2.9)	1.33 (0.39, 4.58)
No (n=593)	135 (22.8)		62 (10.5)		13 (2.2)	
Family history of hay fever						
Yes (n= 72)	18 (25.0)	1.00 (0.66, 1.53)	11 (15.3)	1.29 (0.72, 2.31)	2 (2.8)	1.24 (0.29, 5.33)
No (n= 623)	155 (24.9)		74 (11.9)		14 (2.2)	
Family history of Eczema						
Yes (n=76)	25 (32.9)	1.38 (0.97, 1.95)	15 (19.7)	**1.75 (1.05, 2.89)**	3 (3.9)	1.88 (0.55, 6.45)
No (n=619)	148 (23.9)		70 (11.3)		13 (2.1)	
Smoke exposure						
Yes (n=471)	117 (24.8)	1.00 (0.76, 1.32)	58 (12.3)	1.03 (0.67, 1.57)	11 (2.3)	1.05 (0.37, 2.99)
No (n=225)	56 (24.9)		27 (12.0)		5 (2.2)	
Household crowding						
Yes (n=537)	140 (26.1)	1.26 (0.90, 1.76)	71 (0.13)	1.50 (0.87, 2.59)	14 (2.6)	2.07 (0.48, 9.02)
No (n=159)	33 (20.8)		14 (8.8)		2 (1.3)	
Other children <18 yrs in household						
Yes (n=601)	159 (26.5)	**1.80 (1.09, 2.96)**	78 (13.0)	1.76 (0.84, 3.70)	14 (2.3)	1.11 (0.25, 4.79)
No (n=95)	14 (14.7)		7 (7.4)		2 (2.1)	
Other children <6 yrs in household						
Yes (n=447)	121 (27.1)	1.30 (0.97, 1.72)	61 (13.6)	1.42 (0.91, 2.21)	11 (2.5)	1.23 (0.43, 3.49)
No (n=249)	52 (20.9)		24 (9.6)		5 (2.0)	
Other children <6 yrs in household, in day care						
Yes (n=68)	18 (26.5)	1.07 (0.71, 1.63)	10 (14.7)	1.23 (0.67, 2.27)	4 (5.9)	**3.07 (1.02, 9.28)**
No (n=628)	155 (24.7)		75 (11.9)		12 (1.9)	

Figure 6.5 Adjusted odds ratios for risk factors associated with sunsequent wheeze[1]



[1]Multiple logistic regression models included the following: baseline characteristics associated with subsequent wheeze in the univariate analyses (Table 6.2.A and Table 6.2.B), pathogens detected at medically attended lower respiratory illness in the first 150 days of follow up associated with subsequent wheeze in the univariate analyses (Supplemental Tables 6.4-6.6), and an interaction term for inpatient RSV MALRI and treatment group.

Figure 6.6 Increased risk of subsequent medically attended wheeze in motavizumab participants who had inpatient RSV medically attended respiratory illness in first 150 days of follow up

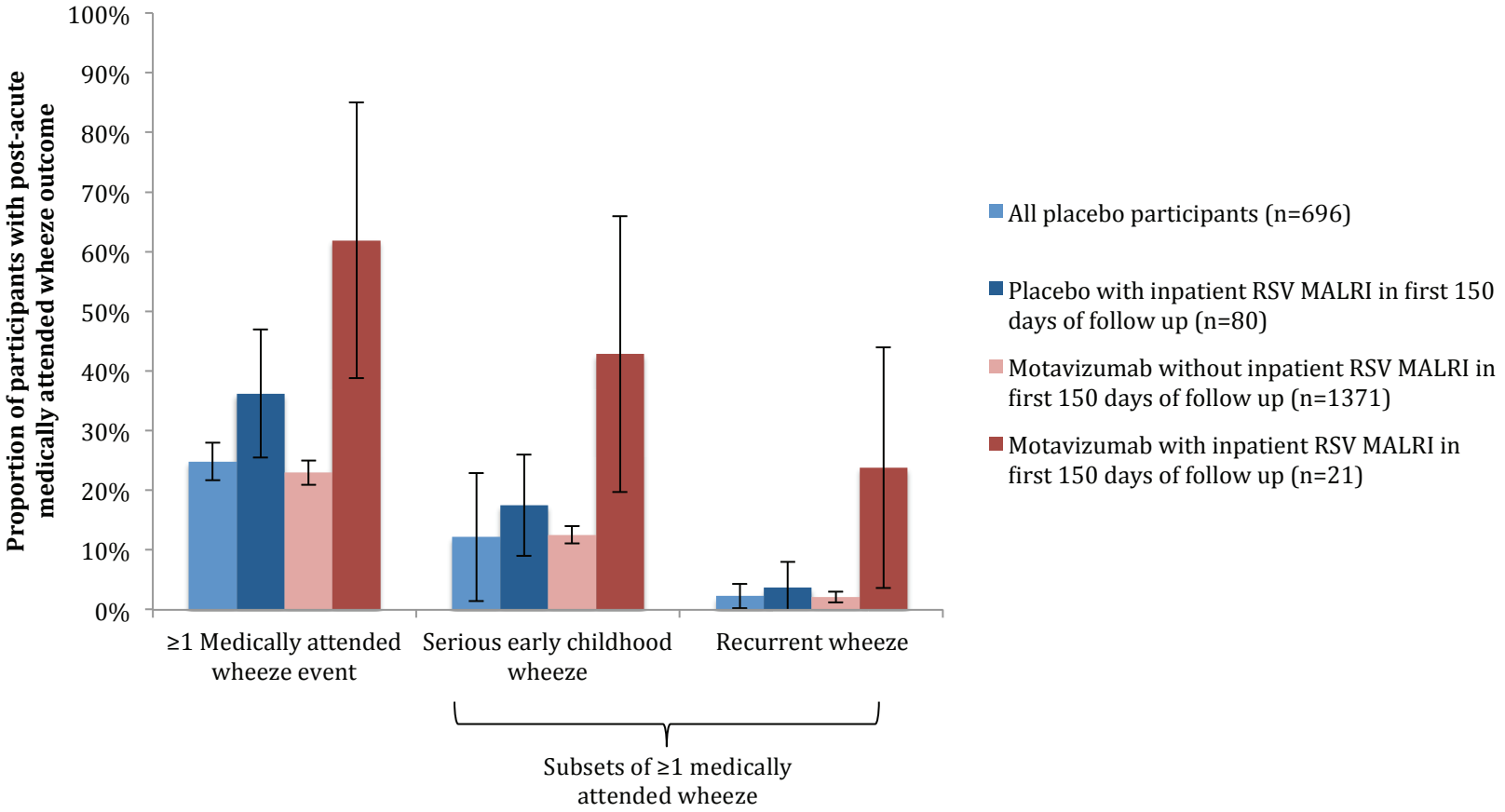
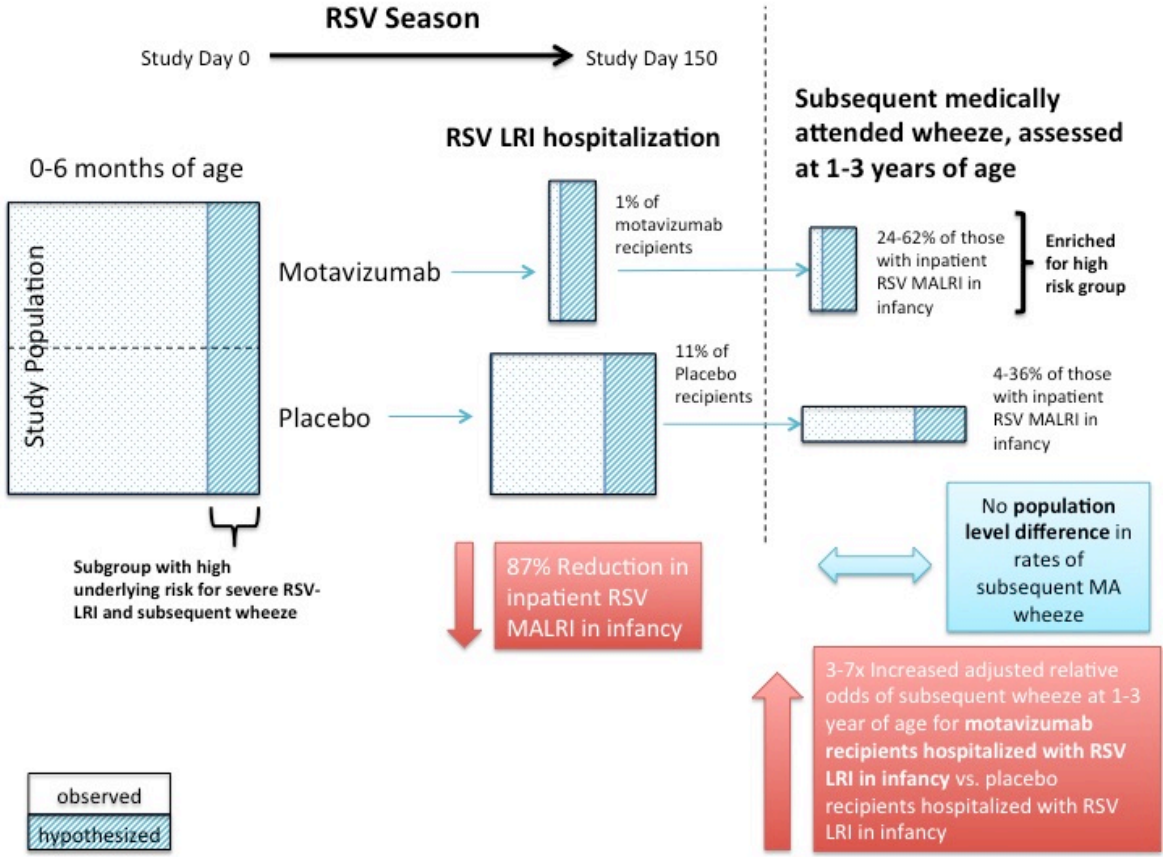


Table 6.4 Characteristics associated with treatment failure (inpatient RSV medically attended respiratory illness in the first 150 days of follow up) in the motavizumab group compared to participants in the motavizumab group without treatment failure, and to placebo recipients with inpatient RSV medically attended respiratory illness in the first 150 days of follow up

	A. Inpatient RSV MALRI, motavizumab group (n=21)	B. No inpatient RSV MALRI, motavizumab group (n=1371)	A vs. B	C. Inpatient RSV MALRI, placebo group (n=80)	A vs. C
	n (%)	**n (%)**	**Relative Risk (95%CI)**	**n (%)**	**Relative Risk (95%CI)**
Male	11 (52.4)	685 (50.0)	1.05 (0.69, 1.58)	33 (41.3)	1.27 (0.78, 2.06)
Attends daycare	2 (9.5)	19 (3.0)	3.30 (0.79, 13.72)	3 (3.8)	2.54 (0.45, 14.29)
Other child <18 in house	21 (100.0)	1191 (86.9)	**1.15 (1.13, 1.17)**	71 (88.8)	**1.13 (1.04, 1.22)**
Other child <6 in house	21 (100.0)	877 (64.0)	**1.56 (1.50, 1.63)**	55 (68.8)	**1.45 (1.25, 1.69)**
Other child <6 in house who attends daycare	6 (28.6)	137 (10.0)	**2.86 (1.43, 5.72)**	8 (10.0)	**2.86 (1.11, 7.34)**
Crowding	20 (95.2)	1068 (78.0)	**1.22 (1.11, 1.35)**	60 (75.0)	**1.27 (1.08, 1.49)**
Smoke exposure	15 (71.4)	942 (68.8)	1.04 (0.79, 1.36)	22 (72.5)	0.99 (0.73, 1.33)
Family history of atopy	10 (47.6)	421 (30.7)	1.55 (0.98, 2.44)	27 (33.6)	1.41 (0.82, 2.43)
Family history of asthma[1]	6 (28.6)	286 (20.9)	1.37 (0.69, 2.71)	14 (17.5)	1.63 (0.71, 3.73)
Family history of wheeze	6 (28.6)	194 (14.2)	**2.02 (1.01, 4.02)**	13 (16.3)	1.75 (0.76, 4.07)
Family history of eczema	3 (14.3)	131 (9.6)	1.49 (0.52, 4.31)	13 (16.3)	0.88 (0.28, 2.80)
Family history of hay fever	3 (14.3)	136 (9.9)	1.44 (0.50, 4.15)	7 (8.8)	1.63 (0.46, 5.78)
Any late or missing study drug	17 (81.0)	633 (46.2)	**1.75 (1.41, 2.17)**	43 (53.8)	**1.51 (1.13, 2.01)**
>1 inpatient MALRI	8 (38.1)	-	-	13 (16.3)	**2.34 (1.12, 4.90)**
Had outpatient MALRI	9 (42.9)	305 (22.2)	**1.93 (1.16, 3.19)**	23 (28.8)	**1.49 (0.82, 2.72)**
RSV detected alone at inpatient RSV MALRI	11 (52.4)	n/a	n/a	44 (55.0)	0.95 (0.61, 1.50)
	Mean (SD)		**p-value**[2]	**Mean (SD)**	**p-value**[2]
Mean number of inpatient MALRI events	1.5 (0.7)	0.1 (0.03)	**<0.01**	1.2 (0.5)	**0.02**
Mean number outpatient MALRI events	0.4 (0.5)	0.3 (0.6)	0.25	0.4 (0.7)	0.59
Age at inpatient RSV MALRI	3.8 (2.3)	n/a	n/a	3.9 (2.1)	0.82

[1]Family history of asthma, wheeze, eczema or hay fever defined as mother, father or sibling having a history of asthma, wheeze, eczema or hay fever.
[2]Student's t-test

Figure 6.7 Motavizumab acts as a probe, revealing sub-group of infants at high risk for both severe RSV-MALRI in infancy and post-acute wheeze at ages 1- 3 years



Supplemental Tables and Figures

Supplemental Table 6.1 Association between inpatient and outpatient medically attended lower respiratory events in the first 150 days of follow up and subsequent medically attended wheeze outcomes

	≥1 medically attended wheeze event			Serious early childhood wheeze			Recurrent wheeze		
Inpatient MALRI	**N (%)**	**OR (95% CI)**	**aOR[1] (95% CI)**	**N (%)**	**OR (95% CI)**	**aOR (95% CI)**	**N (%)**	**OR (95% CI)**	**aOR (95% CI)**
Yes (n=231)	87 (37.7)	**2.11 (1.58, 2.81)**	**2.04 (1.55, 2.74)**	49 (21.2)	**2.07 (1.58, 2.71)**	**2.00 (1.41, 2.84)**	16 (6.9)	**3.78 (2.12, 6.74)**	**4.15 (2.22, 7.74)**
No (n=1857)	414 (22.3)			217 (11.7)			34 (1.8)		
Outpatient MALRI									
Yes (n=511)	177 (34.6)	**2.05 (1.65, 2.55)**	**1.96 (1.57, 2.44)**	100 (19.6)	**2.03 (1.44, 2.88)**	**2.00 (1.51 2.63)**	20 (3.9)	**2.06 (1.18, 3.59)**	**1.92 (1.07, 3.43)**
No (n=1577)	324 (24.0)			166 (10.5)			30 (1.9)		

[1]Adjusted for baseline risk factors for wheeze: family history of asthma, wheeze eczema, hay fever, household crowding, presence of another child <18 yrs, and presence of another child <6 yrs in the household who attends day care.

Supplemental Table 6.2.A Association between virus detected at inpatient medically attended lower respiratory illness in the first 150 days of follow up and ≥1 subsequent medically attended wheeze event

Placebo (n=696)			Motavizumab (n=1,392)			Combined (n=2,088)		
	N(%)	RR (95%CI)		N(%)	RR (95%CI)		N(%)	RR (95%CI)
RSV [1]			**RSV**			**RSV**		
Yes (n=80)	51 (36.3)	**1.55 (1.12, 2.14)**	**Yes (n=21)**	13 (61.9)	**2.69 (1.90, 3.82)**	**Yes (n=101)**	42 (41.5)	**1.80 (1.41, 2.30)**
No (n=616)	472 (23.3)		**No (n=1,371)**	315 (23.0)		**No (n=1,986)**	459 (13.1)	
HRV A			**HRV A**			**HRV A**		
Yes (n=14)	7 (50.0)	**2.05 (1.20, 3.52)**	**Yes (n=18)**	7 (38.9)	1.66 (0.93, 2.99)	**Yes (n=32)**	14 (43.8)	**1.85 (1.24, 2.76)**
No (n=682)	516 (24.3)		**No (n=1,374)**	321 (23.3)		**No (n=2,056)**	487 (23.7)	
HRV B			**HRV B**			**HRV B**		
Yes (n=2)	1 (50.0)	2.02 (0.50, 8.12)	**Yes (n=2)**	0 (0.0)	-	**Yes (n=4)**	1 (25.0)	1.04 (0.19, 5.70)
No (n=694)	522 (24.8)		**No (n=1,390)**	328 (23.6)		**No (n=2,084)**	500 (24.0)	
HRV C			**HRV C**			**HRV C**		
Yes (n=19)	6 (31.6)	1.28 (0.65, 2.51)	**Yes (n=15)**	6 (40.0)	1.71 (0.91, 3.20)	**Yes (n=34)**	12 (35.3)	1.48 (0.93, 2.35)
No (n=677)	167 (24.7)		**No (n=1,377)**	322 (23.4)		**No (n=2054)**	489 (23.8)	
PIV			**PIV**			**PIV**		
Yes (n=16)	9 (56.3)	**2.33 (1.48, 3.67)**	**Yes (n=14)**	6 (57.1)	**2.46 (1.55, 3.91)**	**Yes (n=30)**	17 (56.7)	**2.41 (1.74, 3.33)**
No (n=680)	164 (24.1)		**No (n=1,378)**	320 (23.2)		**No (n=2,058)**	484 (23.5)	
HMPV			**HMPV**			**HMPV**		
Yes (n=12)	4 (33.3)	1.35 (0.60, 3.04)	**Yes (n=12)**	5 (41.7)	1.78 (0.91, 3.50)	**Yes (n=24)**	9 (37.5)	1.57 (0.93, 2.65)
No (n=684)	169 (24.0)		**No (n=1,380)**	323 (23.4)		**No (n=2064)**	492 (23.8)	
Adeno			**Adeno**			**Adeno**		
Yes (n=10)	3 (30.0)	1.21 (0.47, 3.15)	**Yes (n=18)**	5 (27.8)	1.18 (0.56, 2.50)	**Yes (n=28)**	8 (28.6)	1.19 (0.66, 2.16)
No (n=686)	170 (24.8)		**No (n=1,374)**	323 (23.5)		**No (n=2060)**	493 (23.9)	
Flu A			**Flu A**			**Flu A**		
Yes (n=7)	2 (28.6)	1.15 (0.35, 3.74)	**Yes (n=12)**	1 (0.08)	0.35 (0.05, 2.30)	**Yes (n=19)**	3 (15.8)	0.67 (0.23, 1.86)
No (n=689)	171 (24.8)		**No (n=1,380)**	327 (23.7)		**No (n=2069)**	498 (24.1)	
Flu B			**Flu B**			**Flu B**		
Yes (n=1)	1 (100.0)	**4.04 (3.55, 4.60)**	**Yes (n=5)**	1 (20.0)	0.84 (0.15, 4.91)	**Yes (n=6)**	2 (33.3)	1.39 (0.45, 4.32)
No (n=695)	172 (24.7)		**No (n=1387)**	327 (23.8)		**No (n=2082)**	499 (24.0)	
Boca			**Boca**			**Boca**		
Yes (n=2)	2 (100.0)	**4.05 (3.56, 4.62)**	**Yes (n=4)**	2 (50.0)	2.13 (0.80, 5.70)	**Yes (n=6)**	4 (66.7)	**2.79 (1.58, 4.94)**
No (n=694)	171 (24.6)		**No (n=1388)**	326 (23.5)		**No (n=2082)**	497 (23.9)	
Coronavirus			**Coronavirus**			**Coronavirus**		
Yes (n=15)	5 (33.3)	1.35 (0.65, 2.80)	**Yes (n=13)**	5 (38.5)	1.64 (0.82, 3.29)	**Yes (n=28)**	10 (35.7)	1.50 (0.91, 2.48)
No (n=681)	168 (24.7)		**No (n=1379)**	323 (23.4)		**No (n=2060)**	491 (23.8)	
S. pneumoniae			***S. pneumoniae***			***S. pneumoniae***		
Yes (n=71)	25 (35.2)	**1.49 (1.05, 2.10)**	**Yes (n=56)**	20 (35.7)	**1.55 (1.08, 2.23)**	**Yes (n=127)**	45 (35.4)	**1.52 (1.19, 1.95)**
No (n=625)	148 (23.7)		**No (n=1336)**	308 (23.1)		**No (n=1961)**	456 (23.2)	

[1]Interaction by treatment group for risk of wheeze following inpatient RSV MALRI. No interaction by treatment group was found for any other pathogen.
HRV A-C: human rhinovirus subtypes A-C; PIV: any of parainfluenza viruses 1-4; HMPV: human metapneumovirus; Adeno: adenovirus; Flu A: influenza type A; Flu B: influenza type B; Boca: bocavirus; S. pneumoniae: *Streptococcus pneumoniae* carriage in the nasopharynx

Supplemental Table 6.2.B Association between virus detected at outpatient medically attended lower respiratory illness in the first 150 days of follow up and ≥1 subsequent medically attended wheeze event

	Placebo (n=696)			Motavizumab (n=1,392)			Combined (n=2,088)	
	N(%)	**RR (95%CI)**		**N(%)**	**RR (95%CI)**		**N(%)**	**RR (95%CI)**
RSV			**RSV**			**RSV**		
Yes (n=69)	24 (35.8)	**1.46 (1.03, 2.08)**	**Yes (n=40)**	13 (32.5)	1.39 (0.88, 2.20)	**Yes (n=109)**	37 (33.9)	**1.45 (1.10, 1.90)**
No (n=627)	149 (23.8)		**No (n=1351)**	315 (23.3)		**No (n=1978)**	464 (23.5)	
HRV A			**HRV A**			**HRV A**		
Yes (n=45)	16 (35.6)	1.47 (0.97, 2.24)	**Yes (n=57)**	28 (49.1)	**2.19 (1.65, 2.90)**	**Yes (n=102)**	44 (43.1)	**1.87 (1.48, 2.38)**
No (n=651)	157 (24.1)		**No (n=1335)**	300 (22.5)		**No (n=1986)**	457 (23.0)	
HRV B			**HRV B**			**HRV B**		
Yes (n=2)	1 (50.0)		**Yes (n=6)**	2 (33.3)	1.42 (0.46, 4.41)	**Yes (n=8)**	3 (37.5)	1.57 (0.64, 3.84)
No (n=694)	172 (24.8)	2.02 (0.50, 8.12)	**No (n=1386)**	326 (23.5)		**No (n=2080)**	498 (23.9)	
HRV C			**HRV C**			**HRV C**		
Yes (n=37)	23 (62.1)	**2.73 (2.04, 3.64)**	**Yes (n=48)**	17 (35.4)	**1.53 (1.03, 2.27)**	**Yes (n=85)**	40 (47.1)	**2.04 (1.61, 2.60)**
No (n=659)	150 (22.8)		**No (n=1344)**	311 (23.1)		**No (n=2003)**	461 (23.0)	
PIV			**PIV**			**PIV**		
Yes (n=15)	6 (40.0)	1.63 (0.87, 3.07)	**Yes (n=40)**	16 (40.0)	**1.73 (1.17, 2.56)**	**Yes (n=55)**	22 (40.0)	**1.70 (1.22, 2.37)**
No (n=681)	167 (24.5)		**No (n=1352)**	312 (23.1)		**No (n=2033)**	479 (23.6)	
HMPV			**HMPV**			**HMPV**		
Yes (n=11)	3 (27.2)	1.10 (0.42, 2.91)	**Yes (n=34)**	20 (41.2)	**1.78 (1.18, 2.69)**	**Yes (n=45)**	17 (37.8)	**1.59 (1.09, 2.34)**
No (n=685)	170 (24.8)		**No (n=1358)**	314 (23.1)		**No (n=2043)**	484 (23.7)	
Adeno			**Adeno**			**Adeno**		
Yes (n=15)	5 (33.3)	1.35 (0.65, 2.80)	**Yes (n=27)**	13 (48.1)	**2.09 (1.39, 3.12)**	**Yes (n=42)**	18 (42.9)	**1.82 (1.27, 2.60)**
No (n=681)	168 (24.7)		**No (n=1365)**	315 (23.1)		**No (n=2046)**	483 (23.6)	
Flu A			**Flu A**			**Flu A**		
Yes (n=5)	3 (60.0)	2.44 (1.18, 5.05)	**Yes (n=24)**	7 (29.2)	1.24 (0.66, 2.34)	**Yes (n=29)**	10 (34.5)	1.45 (0.87, 2.40)
No (n=691)	170 (24.6)		**No (n=1368)**	321 (23.5)		**No (n=2059)**	491 (23.8)	
Flu B			**Flu B**			**Flu B**		
Yes (n=5)	2 (40.0)	1.62 (0.55, 4.77)	**Yes (n=9)**	4 (44.4)	1.90 (0.91, 3.96)	**Yes (n=14)**	6 (42.9)	1.80 (0.98, 3.30)
No (n=691)	171 (24.7)		**No (n=1383)**	324 (23.4)		**No (n=2074)**	495 (23.9)	
Boca			**Boca**			**Boca**		
Yes (n=9)	3 (33.3)	1.35 (0.53, 3.42)	**Yes (n=16)**	8 (50.0)	**2.15 (1.30, 3.54)**	**Yes (n=25)**	11 (44.0)	**1.85 (1.18, 2.90)**
No (n=687)	170 (24.7)		**No (n=1376)**	320 (23.3)		**No (n=2063)**	490 (23.8)	
Coronavirus			**Coronavirus**			**Coronavirus**		
Yes (n=13)	4 (30.7)	1.24 (0.54, 2.84)	**Yes (n=47)**	17 (36.2)	**1.56 (1.06, 2.32)**	**Yes (n=60)**	21 (35.0)	**1.48 (1.04, 2.11)**
No (n=683)	169 (24.7)		**No (n=1345)**	311 (23.1)		**No (n=2028)**	480 (23.7)	

S. pneumoniae			***S. pneumoniae***			***S. pneumoniae***		
Yes (n=98)	33 (33.7)	**1.44 (1.05, 1.97)**	**Yes (n=153)**	61 (39.9)	**1.85 (1.48, 2.31)**	**Yes (n=251)**	94 (37.5)	**1.69 (1.41, 2.03)**
No (n=598)	140 (23.4)		**No (n=1239)**	267 (21.5)		**No (n=1837)**	407 (22.2)	

HRV A-C: human rhinovirus subtypes A-C; PIV: any of parainfluenza viruses 1-4; HMPV: human metapneumovirus; Adeno: adenovirus; Flu A: influenza type A; Flu B: influenza type B; Boca: bocavirus; S. pneumoniae: *Streptococcus pneumoniae* carriage in the nasopharynx.

Supplemental Table 6.3.A Association between virus detected at inpatient medically attended lower respiratory illness in the first 150 days of follow up and subsequent medically attended serious early childhood wheeze

	Placebo (n=696)			Motavizumab (n=1,392)			Combined (n=2,088)	
	N(%)	**RR (95%CI)**		**N(%)**	**RR (95%CI)**		**N(%)**	**RR (95%CI)**
RSV (+)1			**RSV (+)**			**RSV (+)**		
Yes (n=80)	14 (17.5)	1.52 (0.90, 2.56)	**Yes (n=21)**	9 (0.43)	**3.41 (2.04, 5.70)**	**Yes (n=101)**	23 (22.8)	**1.86 (1.28, 2.72)**
No (n=616)	71 (11.5)		**No (n=1370)**	172 (12.6)		**No (n=1986)**	243 (12.2)	
HRV (+)			**HRV (+)**			**HRV (+)**		
Yes (n=32)	6 (18.8)	1.58 (0.74, 3.34)	**Yes (n=32)**	7 (2.19)	1.71 (0.88, 3.34)	**Yes (n=64)**	13 (20.2)	1.63 (0.99, 2.68)
No (n=664)	79 (11.9)		**No (n=1360)**	174 (12.8)		**No (n=2024)**	253 (12.5)	
HRV A (+)			**HRV A (+)**			**HRV A (+)**		
Yes (n=14)	4 (28.6)	**2.41 (1.03, 5.65)**	**Yes (n=18)**	3 (16.7)	1.29 (0.45, 3.65)	**Yes (n=32)**	7 (21.9)	1.74 (0.89, 3.38)
No (n=682)	81 (11.9)		**No (n=1374)**	178 (13.0)		**No (n=2056)**	259 (12.6)	
HRV B (+)			**HRV B (+)**			**HRV B (+)**		
Yes (n=2)	0 (0.0)	-	**Yes (n=2)**	0 (0.0)	-	**Yes (n=4)**	0 (0.0)	-
No (n=694)	85 (12.2)		**No (n=1390)**	181 (13.0)		**No (n=2084)**	266 (12.8)	
HRV C (+)			**HRV C (+)**			**HRV C (+)**		
Yes (n=19)	3 (15.8)	1.30 (0.45, 3.76)	**Yes (n=15)**	4 (26.7)	2.07 (0.89, 4.86)	**Yes (n=34)**	7 (20.6)	1.63 (0.84, 3.19)
No (n=677)	82 (12.1)		**No (n=1377)**	177 (12.9)		**No (n=2054)**	259 (12.6)	
PIV (+)			**PIV (+)**			**PIV (+)**		
Yes (n=16)	6 (37.5)	**3.23 (1.66, 6.28)**	**Yes (n=14)**	4 (28.6)	**2.22 (0.96, 5.15)**	**Yes (n=30)**	10 (33.3)	**2.68 (1.59, 4.50)**
No (n=680)	79 (11.6)		**No (n=1378)**	177 (12.8)		**No (n=2058)**	256 (12.4)	
HMPV (+)			**HMPV (+)**			**HMPV (+)**		
Yes (n=12)	1 (0.08)	0.68 (0.10, 4.48)	**Yes (n=12)**	1 (0.08)	0.64 (0.10, 4.19)	**Yes (n=24)**	2 (0.08)	0.65 (0.17, 2.47)
No (n=684)	84 (12.3)		**No (n=1380)**	180 (13.0)		**No (n=2064)**	264 (12.7)	
Adeno (+)			**Adeno (+)**			**Adeno (+)**		
Yes (n=10)	2 (20.0)	1.65 (0.47, 5.80)	**Yes (n=18)**	5 (27.8)	2.17 (1.02, 4.63)	**Yes (n=28)**	7 (25.0)	**1.99 (1.04, 3.81)**
No (n=686)	83 (12.1)		**No (n=1374)**	176 (12.8)		**No (n=2060)**	259 (12.6)	
Flu A (+)			**Flu A (+)**			**Flu A (+)**		
Yes (n=7)	2 (28.6)	2.37 (0.72, 7.78)	**Yes (n=12)**	1 (0.08)	0.64, (0.10, 4.19)	**Yes (n=19)**	3 (15.8)	1.24 (0.44, 3.53)
No (n=689)	83 (12.0)		**No (n=1380)**	180 (13.0)		**No (n=2069)**	263 (12.7)	
Flu B (+)			**Flu B (+)**			**Flu B (+)**		
Yes (n=1)	0 (0.0)	-	**Yes (n=5)**	1 (20.0)	1.54 (0.27, 8.84)	**Yes (n=6)**	1 (16.7)	1.31 (0.22, 7.86)
No (n=695)	85 (12.2)		**No (n=1387)**	180 (13.0)		**No (n=2082)**	256 (12.7)	
Boca (+)			**Boca (+)**			**Boca (+)**		
Yes (n=2)	0 (0.0)	-	**Yes (n=4)**	1 (25.0)	1.93 (0.35, 10.58)	**Yes (n=6)**	1 (16.7)	1.31 (0.22, 7.86)
No (n=694)	85 (12.2)		**No (n=1388)**	180 (13.0)		**No (n=2082)**	265 (12.7)	
Coronavirus			**Coronavirus**			**Coronavirus**		
Yes (n=15)	3 (20.0)	1.66 (0.59, 4.66)	**Yes (n=13)**	3 (23.0)	1.79 (0.66, 4.87)	**Yes (n=28)**	6 (21.4)	1.70 (0.82, 3.48)
No (n=681)	82 (12.0)		**No (n=1379)**	178 (12.9)		**No (n=2060)**	260 (12.6)	
S. pneumoniae			***S. pneumoniae***			***S. pneumoniae***		
Yes (n=71)	11 (15.5)	1.31 (0.73, 2.35)	**Yes (n=56)**	13 (23.2)	**1.85 (1.12, 3.30)**	**Yes (n=127)**	24 (18.9)	**1.53 (1.05, 2.24)**
No (n=625)	74 (11.8)		**No (n=1336)**	168 (12.6)		**No (n=1961)**	242 (12.3)	

1Interaction by treatment group for risk of wheeze following inpatient RSV MALRI. No interaction by treatment group was found for any other pathogen.
HRV A-C: human rhinovirus subtypes A-C; PIV: any of parainfluenza viruses 1-4; HMPV: human metapneumovirus; Adeno: adenovirus; Flu A: influenza type A; Flu B: influenza type B; Boca: bocavirus; S. pneumoniae: *Streptococcus pneumoniae* carriage in the nasopharynx.

Supplemental Table 6.3.B Association between virus detected at outpatient medically attended lower respiratory illness in the first 150 days of follow up and subsequent medically attended serious early childhood wheeze

	Placebo (n=696)			Motavizumab (n=1,392)			Combined (n=2,088)	
	N(%)	**RR (95%CI)**		**N(%)**	**RR (95%CI)**		**N(%)**	**RR (95%CI)**
RSV (+)			**RSV (+)**			**RSV (+)**		
Yes (n=69)	12 (17.4)	1.49 (0.86, 2.61)	**Yes (n=40)**	8 (20.0)	1.56 (0.83, 2.95)	**Yes (n=109)**	20 (18.3)	1.48 (0.98, 2.23)
No (n=627)	73 (11.6)		**No (n=1351)**	173 (12.8)		**No (n=1978)**	246 (12.4)	
HRV A (+)			**HRV A (+)**			**HRV A (+)**		
Yes (n=45)	11 (24.4)	**2.15 (1.23, 3.75)**	**Yes (n=57)**	17 (29.8)	**2.43 (1.59, 3.71)**	**Yes (n=102)**	28 (27.5)	**2.29 (1.63, 3.21)**
No (n=651)	74 (11.4)		**No (n=1335)**	164 (12.3)		**No (n=1986)**	238 (12.0)	
HRV B (+)			**HRV B (+)**			**HRV B (+)**		
Yes (n=2)	1 (50.0)	**4.13 (1.02, 16.76)**	**Yes (n=6)**	2 (33.3)	2.58 (0.83, 8.07)	**Yes (n=8)**	3 (37.5)	**2.97 (1.20, 7.31)**
No (n=694)	84 (12.1)		**No (n=1386)**	179 (12.9)		**No (n=2080)**	263 (12.6)	
HRV C (+)			**HRV C (+)**			**HRV C (+)**		
Yes (n=37)	15 (40.5)	**3.82 (2.44, 5.98)**	**Yes (n=48)**	10 (20.8)	1.64 (0.93, 2.89)	**Yes (n=85)**	25 (29.4)	**2.44 (1.72, 3.47)**
No (n=659)	70 (10.6)		**No (n=1344)**	171 (12.7)		**No (n=2003)**	241 (12.0)	
PIV (+)			**PIV (+)**			**PIV (+)**		
Yes (n=15)	2 (13.3)	1.09 (0.30, 4.04)	**Yes (n=40)**	70 (25.0)	**1.98 (1.14, 3.44)**	**Yes (n=55)**	12 (21.8)	**1.75 (1.05, 2.92)**
No (n=681)	83 (12.2)		**No (n=1352)**	171 (12.6)		**No (n=2033)**	254 (12.5)	
HMPV (+)			**HMPV (+)**			**HMPV (+)**		
Yes (n=11)	2 (18.2)	1.50 (0.42, 5.34)	**Yes (n=34)**	7 (20.6)	1.61 (0.82, 3.15)	**Yes (n=45)**	9 (20.0)	1.59 (0.88, 2.88)
No (n=685)	83 (12.1)		**No (n=1358)**	174 (12.8)		**No (n=2043)**	257 (12.6)	
Adeno (+)			**Adeno (+)**			**Adeno (+)**		
Yes (n=15)	4 (26.7)	2.24 (0.95, 5.32)	**Yes (n=27)**	8 (29.6)	**2.34 (1.29, 4.25)**	**Yes (n=42)**	12 (16.2)	**2.30 (1.41, 3.76)**
No (n=681)	81 (11.9)		**No (n=1365)**	173 (12.7)		**No (n=2046)**	254 (12.4)	
Flu A (+)			**Flu A (+)**			**Flu A (+)**		
Yes (n=5)	1 (20.0)	1.65 (0.28, 9.61)	**Yes (n=24)**	5 (20.8)	1.62 (0.73, 3.58)	**Yes (n=29)**	6 (20.7)	1.64 (0.80, 3.37)
No (n=691)	84 (12.1)		**No (n=1368)**	176 (12.9)		**No (n=2059)**	260 (12.6)	
Flu B (+)			**Flu B (+)**			**Flu B (+)**		
Yes (n=5)	1 (20.0)	**1.65 (0.28, 9.61)**	**Yes (n=9)**	1 (11.1)	**2.95 (1.54, 5.63)**	**Yes (n=14)**	2 (14.3)	1.12, (0.31, 4.07)
No (n=691)	84 (12.2)		**No (n=1383)**	180 (13.0)		**No (n=2074)**	264 (12.7)	
Boca (+)			**Boca (+)**			**Boca (+)**		
Yes (n=9)	1 (11.1)	0.91 (0.14, 5.83)	**Yes (n=16)**	6 (37.5)	**2.94 (1.54, 5.63)**	**Yes (n=25)**	7 (28.0)	**2.23 (1.18, 4.22)**
No (n=687)	84 (12.2)		**No (n=1376)**	175 (12.7)		**No (n=2063)**	259 (12.6)	
Coronavirus			**Coronavirus**			**Coronavirus**		
Yes (n=13)	1 (15.3)	1.27 (0.35, 4.60)	**Yes (n=47)**	11 (23.4)	**1.85 (1.08, 3.16)**	**Yes (n=60)**	13 (21.7)	**1.73 (1.06, 2.85)**
No (n=683)	83 (12.2)		**No (n=1345)**	170 (12.6)		**No (n=2028)**	253 (12.5)	
S. pneumoniae			***S. pneumoniae***			***S. pneumoniae***		
Yes (n=98)	19 (19.4)	**1.76 (1.11, 2.79)**	**Yes (n=153)**	37 (24.2)	**2.08 (1.51, 2.86)**	**Yes (n=251)**	56 (22.3)	**1.95 (1.50, 2.54)**
No (n=598)	66 (11.0)		**No (n=1239)**	144 (11.6)		**No (n=1837)**	210 (11.4)	

HRV A-C: human rhinovirus subtypes A-C; PIV: any of parainfluenza viruses 1-4; HMPV: human metapneumovirus; Adeno: adenovirus; Flu A: influenza type A; Flu B: influenza type B; Boca: bocavirus; S. pneumoniae: *Streptococcus pneumoniae* carriage in the nasopharynx.

Supplemental Table 6.4.A Association between virus detected at inpatient medically attended lower respiratory illness in the first 150 days of follow up and subsequent medically attended recurrent wheeze

	Placebo (n=696)			Motavizumab (n=1,392)			Combined (n=2,088	
	N(%)	**RR (95%CI)**		**N(%)**	**RR (95%CI)**		**N(%)**	**RR (95%CI)**
RSV (+)[1]			**RSV (+)**			**RSV (+)**		
Yes (n=80)	3 (3.8)	1.78 (0.52, 6.10)	**Yes (n=21)**	5 (23.8)	**11.25 (4.83, 26.20)**	**Yes (n=101)**	8 (7.9)	**3.75 (1.81, 7.77)**
No (n=616)	13 (2.1)		**No (n=1370)**	29 (2.1)		**No (n=1986)**	42 (2.1)	
HRV A (+)			**HRV A (+)**			**HRV A (+)**		
Yes (n=14)	3 (21.4)	**11.24 (3.60, 35.09)**	**Yes (n=18)**	1 (5.6)	2.31 (0.33, 16.01)	**Yes (n=32)**	4 (12.5)	**6.24 (2.10, 18.52)**
No (n=682)	13 (1.9)		**No (n=1374)**	33 (2.4)		**No (n=2056)**	46 (2.2)	
HRV B (+)			**HRV B (+)**			**HRV B (+)**		
Yes (n=2)	0 (0.0)	-	**Yes (n=2)**	0 (0.0)	-	**Yes (n=4)**	0 (0.0)	-
No (n=694)	16 (2.3)		**No (n=1390)**	34 (2.4)		**No (n=2084)**	50 (2.4)	
HRV C (+)			**HRV C (+)**			**HRV C (+)**		
Yes (n=19)	1 (5.3)	2.40 (0.33, 17.07)	**Yes (n=15)**	1 (6.7)	2.78 (0.41, 19.04)	**Yes (n=34)**	2 (5.9)	2.61 (0.61, 11.21)
No (n=677)	15 (2.2)		**No (n=1377)**	33 (2.4)		**No (n=2054)**	48 (2.3)	
PIV (+)			**PIV (+)**			**PIV (+)**		
Yes (n=16)	2 (12.5)	**6.07 (1.50, 24.53)**	**Yes (n=14)**	1 (7.1)	2.98 (0.44, 20.31)	**Yes (n=30)**	3 (10.0)	**4.38 (1.44, 13.29)**
No (n=680)	14 (2.1)		**No (n=1378)**	33 (2.4)		**No (n=2058)**	47 (2.3)	
HMPV (+)			**HMPV (+)**			**HMPV (+)**		
Yes (n=12)	1 (8.3)	3.80 (0.54, 26.50)	**Yes (n=12)**	0 (0.0)	-	**Yes (n=24)**	1 (4.2)	1.76 (0.25, 12.20)
No (n=684)	15 (2.2)		**No (n=1380)**	34 (2.5)		**No (n=2064)**	49 (2.4)	
Adeno (+)			**Adeno (+)**			**Adeno (+)**		
Yes (n=10)	0 (0.0)	-	**Yes (n=18)**	0 (0.0)	-	**Yes (n=28)**	0 (0.0)	-
No (n=686)	16 (2.3)		**No (n=1374)**	34 (2.5)		**No (n=2060)**	50 (2.4)	
Flu A (+)			**Flu A (+)**			**Flu A (+)**		
Yes (n=7)	0 (0.0)	-	**Yes (n=12)**	0 (0.0)		**Yes (n=19)**	0 (0.0)	-
No (n=689)	16 (2.3)		**No (n=1380)**	34 (2.5)		**No (n=2069)**	50 (2.4)	
Flu B (+)			**Flu B (+)**			**Flu B (+)**		
Yes (n=1)	0 (0.0)	-	**Yes (n=5)**	1 (20.0)	8.41 (1.41, 50.10)	**Yes (n=6)**	1 (16.7)	**7.08 (1.16, 43.29)**
No (n=695)	16 (2.3)		**No (n=1387)**	33 (2.4)		**No (n=2082)**	49 (2.4)	
Boca (+)			**Boca (+)**			**Boca (+)**		
Yes (n=2)	0 (0.0)	-	**Yes (n=4)**	0 (0.0)	-	**Yes (n=6)**	0 (0.0)	-
No (n=694)	16 (2.3)		**No (n=1388)**	34 (2.4)		**No (n=2082)**	50 (2.4)	
Coronavirus			**Coronavirus**			**Coronavirus**		
Yes (n=15)	1 (6.3)	3.03 (0.43, 21.71)	**Yes (n=13)**	1 (7.7)	3.21 (0.47, 21.77)	**Yes (n=6)**	2 (7.1)	3.07 (0.78, 12.00)
No (n=681)	14 (2.1)		**No (n=1379)**	33 (2.4)		**No (n=2082)**	48 (2.3)	
S. pneumoniae			***S. pneumoniae***			***S. pneumoniae***		
Yes (n=71)	3 (4.2)	2.03 (0.59, 6.96)	**Yes (n=56)**	4 (7.1)	**3.18 (1.16, 8.72)**	**Yes (n=127)**	7 (5.5)	**2.51 (1.15, 5.47)**
No (n=625)	13 (2.1)		**No (n=1336)**	30 (2.2)		**No (n=1961)**	43 (2.2)	

[1]Interaction by treatment group for risk of wheeze following inpatient RSV MALRI. No interaction by treatment group was found for any other pathogen.
HRV A-C: human rhinovirus subtypes A-C; PIV: any of parainfluenza viruses 1-4; HMPV: human metapneumovirus; Adeno: adenovirus; Flu A: influenza type A; Flu B: influenza type B; Boca: bocavirus; S. pneumoniae: *Streptococcus pneumoniae* carriage in the nasopharynx.

Supplemental Table 6.4.B Association between virus detected at outpatient medically attended lower respiratory illness in the first 150 days of follow up and subsequent medically attended recurrent wheeze

	Placebo (n=696)			Motavizumab (n=1,392)			Combined (n=2,088)	
	N(%)	**RR (95%CI)**		**N(%)**	**RR (95%CI)**		**N(%)**	**RR (95%CI)**
RSV (+)			**RSV (+)**			**RSV (+)**		
Yes (n=69)	1 (6.3)	0.61 (0.08, 4.52)	**Yes (n=40)**	1 (2.5)	1.02 (0.14, 7.30)	**Yes (n=109)**	2 (1.8)	0.76 (0.19, 3.07)
No (n=627)	15 (2.4)		**No (n=1351)**	33 (2.4)		**No (n=1978)**	48 (2.4)	
HRV A (+)			**HRV A (+)**			**HRV A (+)**		
Yes (n=45)	3 (6.7)	3.34 (0.99, 11.29)	**Yes (n=57)**	3 (5.3)	2.27 (0.71, 7.19)	**Yes (n=102)**	6 (5.9)	**2.66 (1.16, 6.09)**
No (n=651)	13 (2.0)		**No (n=1335)**	31 (2.3)		**No (n=1986)**	44 (2.2)	
HRV B (+)			**HRV B (+)**			**HRV B (+)**		
Yes (n=2)	0 (0.0)	-	**Yes (n=6)**	0 (0.0)	-	**Yes (n=8)**	0 (0.0)	-
No (n=694)	16 (2.3)		**No (n=1386)**	34 (2.5)		**No (n=2080)**	50 (2.4)	
HRV C (+)			**HRV C (+)**			**HRV C (+)**		
Yes (n=37)	3 (8.1)	**4.11 (1.22, 13. 80**)	**Yes (n=48)**	1 (2.1)	0.85 (0.12, 6.08)	**Yes (n=85)**	4 (4.7)	2.05 (0.76, 5.56)
No (n=659)	13 (2.0)		**No (n=1344)**	33 (2.5)		**No (n=2003)**	46 (2.3)	
PIV (+)			**PIV (+)**			**PIV (+)**		
Yes (n=15)	0 (0.0)	-	**Yes (n=40)**	3 (7.5)	**3.27 (1.04, 10.25)**	**Yes (n=55)**	3 (5.5)	**2.36 (0.76, 7.35)**
No (n=681)	16 (2.3)		**No (n=1352)**	31 (2.3)		**No (n=2033)**	47 (2.3)	
HMPV (+)			**HMPV (+)**			**HMPV (+)**		
Yes (n=11)	0 (0.0)	-	**Yes (n=34)**	2 (5.9)	2.50 (0.62, 10.00)	**Yes (n=45)**	2 (4.4)	1.89 (0.47, 7.54)
No (n=685)	16 (2.3)		**No (n=1358)**	32 (2.4)		**No (n=2043)**	48 (2.3)	
Adeno (+)			**Adeno (+)**			**Adeno (+)**		
Yes (n=15)	2 (13.3)	**6.49 (1.61, 26.05)**	**Yes (n=27)**	1 (3.7)	1.53 (0.22, 10.80)	**Yes (n=42)**	3 (7.1)	**3.11 (1.01, 9.59)**
No (n=681)	14 (2.1)		**No (n=1365)**	33 (2.4)		**No (n=2046)**	47 (2.3)	
Flu A (+)			**Flu A (+)**			**Flu A (+)**		
Yes (n=5)	0 (0.0)	-	**Yes (n=24)**	1 (4.2)	1.73 (0.25, 12.12)	**Yes (n=29)**	1 (3.4)	1.45 (0.21, 10.14)
No (n=691)	16 (2.3)		**No (n=1368)**	33 (2.4)		**No (n=2059)**	49 (2.4)	
Flu B (+)			**Flu B (+)**			**Flu B (+)**		
Yes (n=5)	0 (0.0)	-	**Yes (n=9)**	0 (0.0)	-	**Yes (n=14)**	0 (0.0)	-
No (n=691)	16 (2.3)		**No (n=1383)**	34 (2.5)		**No (n=2074)**	50 (2.4)	
Boca (+)			**Boca (+)**			**Boca (+)**		
Yes (n=9)	0 (0.0)	-	**Yes (n=16)**	1 (6.3)	2.61 (0.38, 17.91)	**Yes (n=25)**	1 (4.0)	
No (n=687)	16 (2.3)		**No (n=1376)**	33 (2.4)		**No (n=2063)**	49 (2.4)	
Coronavirus			**Coronavirus**			**Coronavirus**		1.68 (0.24, 11.72)
Yes (n=13)	0 (0.0)	-	**Yes (n=47)**	5 (10.6)	**4.93 (2.00, 12.18)**	**Yes (n=60)**	5 (8.3)	**3.76 (1.55, 9.12)**
No (n=683)	16 (2.3)		**No (n=1345)**	29 (2.2)		**No (n=2028)**	45 (2.2)	
S. pneumoniae			***S. pneumoniae***			***S. pneumoniae***		
Yes (n=98)	3 (3.1)	1.41 (0.41, 4.85)	**Yes (n=153)**	10 (6.5)	**3.37 (1.65, 6.92)**	**Yes (n=251)**	13 (5.2)	**2.57 (1.39, 4.77)**
No (n=598)	13 (2.2)		**No (n=1239)**	24 (1.9)		**No (n=1837)**	37 (2.0)	

*Interaction by treatment group for risk of wheeze following inpatient RSV MALRI. No interaction by treatment group was found for any other pathogen.
HRV A-C: human rhinovirus subtypes A-C; PIV: any of parainfluenza viruses 1-4; HMPV: human metapneumovirus; Adeno: adenovirus; Flu A: influenza type A; Flu B: influenza type B; Boca: bocavirus; S. pneumoniae: *Streptococcus pneumoniae* carriage in the nasopharynx.

Supplemental Table 6.5 Medically attended acute lower respiratory illness events in the first 150 days of follow up in participants with motavizumab treatment failure[1]

		Inpatient MALRI[2]								Outpatient MALRI	
		First inpatient MALRI				Second inpatient MALRI					
ID	No. of events	Age (mo)	Viruses detected	Study day	Days since last study drug dose if (*)	Age (mo)	Viruses detected	Study day	Days since last study drug dose if (*)	No. of events	Viruses detected
1*	1	1.5	RSV A	29	29	-	-	-	-	0	-
2*	1	1.7	RSV A	36	10	-	-	-	-	0	-
3*	1	1.2	RSV A	36	36	-	-	-	-	0	-
4*	1	1.4	RSV A	41	16	-	-		-	0	-
5*	1	6.7	RSV A	116	31	-	-	-	-	1	No specimen in analytic window
6*	1	5.8	RSV A	41	19	-	-	-	-	1	PIV-3
7*	1	9.9	RSV B[3]	118	10	-	-	-	-	0	-
8*	1	5.1	RSV B	103	33	-	-	-	-	1	HRV C, CoV NL63
9*	1	6.9	RSVA, Adeno	51	22	-	-	-	-	1	No specimen in analytic window
10*	1	4.4	RSVA, PIV-4, Cov NL63	60	34	-	-	-	-	0	-
11*	1	3.1	RSV A, Boca	92	28	-	-		-	0	-
12*	1	5.9	RSV B, Flu A	51	51[d]	-	-	-	-	0	-
13*	1	2.5	RSV A, Adeno	60	4	-	-	-	-	0	-
14	2	1.2	No specimen in analytic window	36	-	2.0	RSVA	58	28	1	RSV B, HRV A
15*	2	2.2	RSV B	49	49	5.3	PIV 4	141	126[4]	1	PIV-4
16*	2	4.3	Negative all viruses	34	34	5.5	RSV B, HRV C	69	69[4]	0	-
17*	2	2.3	RSV B	68	28	3.3	RSV negative[3]	98	58[4]	1	No specimen in analytic window
18	2	1.5	Negative all viruses	44	12	2.4	RSV B, HMPV Adeno	69	16	0	-
19	2	1.9	RSV A	49	-	3.7	HRV C	101	-	1	HRV A
20*	3	4.2	HRV C, Adeno	77	22	4.6	RSV A, HRV C, Adeno	89	-	1	HRV A, Adv, Boca
20 -3rd inpatient MALRI		6.4	HMPV	126	3	-	-	-	-	-	-
21	3	1.0	RSV-negative[3]	113	-	4.2	RSV A[3]	-	-	0	-
21 – 3rd inpatient MALRI		4.4	RSV A[3]	-	-	-	-	-	-	-	-

*Participant had at least one late or missed dose of study drug during the trial

[1]Treatment failure defined as one or more inpatient RSV medically attended lower respiratory illness

[2]Medically attended lower respiratory illness
[3]Sample not available for additional viral testing
[4]Plus at least one missed dose before this medically attended lower respiratory illness
HRV A-C: human rhinovirus subtypes A-C; Cov: coronavirus; PIV: any of parainfluenza viruses 1-4; HMPV: human metapneumovirus; Adeno: adenovirus; Flu A: influenza type A; Flu B: influenza type B; Boca: bocavirus; S. pneumoniae: *Streptococcus pneumoniae* carriage in the nasopharynx.

Chapter 7: Evaluating the risk of respiratory syncytial virus lower respiratory illness in the second RSV season after disease prevention with immunoprophylaxis in the first season

Abstract

Background:

Respiratory syncytial virus (RSV) associated lower respiratory illness is a leading cause of child morbidity and is most severe in early infancy. To what extent this is attributable to young age and to what extent it is due to the experience of a primary infection is not fully understood. Recently, a phase three randomized trial of a next-generation RSV monoclonal antibody, motavizumab, was shown to have high efficacy for the prevention of inpatient and outpatient RSV-associated medically attended lower respiratory illness (MALRI) in a population of healthy full term infants during their first winter RSV season. We assessed whether there was increased risk of RSV MALRI in this population in the second RSV season following RSV MALRI prevention in the first season.

Methods:

Infants less than six months of age by December 31st of any of the four years of enrollment in the motavizumab trial were enrolled and prophylaxed with monthly doses of motavizumab or placebo for five total doses through the first winter RSV season (150 days following randomization). Nasopharyngeal samples were collected at every MALRI for three years following enrollment in the motavizumab trial. We tested stored nasopharyngeal samples collected at MALRI events that occurred during the RSV season of the second year of life for RSV A and B using real-time PCR.

Results:

We observed no increased relative risk of RSV-MALRI events in the second season for the motavizumab group compared to the placebo group (RR 1.09 (95%CI 0.79, 1.50, equivalent to a <1% increase in absolute risk). Participants with RSV MALRI in the first RSV season were less likely to have an RSV MALRI, but more likely to have a non-RSV MALRI in the second RSV season compared to participants with no RSV MALRI in the first season.

Discussion:

We found no statistical difference in rates of medically attended RSV illness, either inpatient or outpatient or both, by treatment group in the second RSV season. This reassures that there is not a substantial increased risk of medially attended respiratory events attributable to RSV in the second year of life among children who had protection against RSV disease as infants. We did observe a 9% relative increase in the rate of any RSV MALRI in the second RSV season for the motavizumab treatment group compared to the placebo treatment group that was not statistically significant, and which corresponded to a <1% absolute increase in the rate of any RSV MALRI. We also observed a trend of decreased severity of RSV MALRI in the second season compared to the first season for both treatment groups. The proportion of MALRI events in the second RSV season with samples collected within the analytic window that were available for RSV testing reduced our statistical power to detect true differences in rates of RSV MALRI between treatment groups in this time period. However, the small magnitude of

the increase in risk that we observed in the motavizumab group, combined with less severe RSV MALRI in the second compared to the first year of life, provides strong support for the benefit of delaying primary RSV lower respiratory illness beyond infancy.

Conclusion:

Young age and the experience of a primary infection are both thought to contribute to elevated risk of severe illness with RSV in infancy. We found no significant increase in RSV MALRI in the second RSV season after preventing RSV MALRI with motavizumab in the first RSV season. Our results support the argument of a significant overall public health benefit to delaying the primary lower respiratory illness until the second year of life.

Introduction

Respiratory syncytial virus (RSV) is a leading cause of child morbidity, with approximately 3.2 million annual hospital admissions and 59,600 – 118,200 deaths in children less than five years of age in 2015 [29]. The burden of severe RSV illness is disproportionately borne by infants less than six months of age, who are estimated to make up 45% of RSV-associated hospital admissions and deaths among children [29]. While RSV-ALRI incidence peaks at ages 3-5 months, it remains elevated during the second year of life before declining thereafter [29]. By two years of age, more than 80% of children have experienced an RSV infection, the majority of which are mild upper airway illnesses, with two thirds of primary infections occurring in the first year [12]. Protection against subsequent episodes of infection and disease following a primary RSV infection is limited and wanes over time [38], with a unique characteristic of the virus being its ability to cause re-infection throughout the lifetime [12, 38, 286, 287]. The precise timeframe of the protective period following natural infection is not completely understood, but has been estimated to be approximately 6 months in children [38].

Young infants are at highest risk of developing severe disease with RSV-infection, but it is not fully understood to what extent this can be attributed to the immaturity of their immune system, anatomical factors, or to the experience of a primary infection [32]. Studies comparing the severity of primary versus subsequent RSV infections in children provide conflicting information and can be confounded by age. Some have found subsequent reinfections to be less severe than primary infections [12], while others have found no difference [38, 39]. If a substantial fraction of the risk of severe RSV illness is

attributed to the experience of the primary infection, regardless of age at which it occurs, we would expect to see an increased incidence of severe RSV-illness in children whose primary infection is delayed beyond infancy compared to those with a primary infection in infancy, controlling for all other factors. This has implications for future immunization strategies that prevent RSV-illness early in life but do not offer sustained protection through childhood. One method to assess the relative contribution of primary infection to the frequency and severity of RSV lower respiratory illness is to compare illness outcomes in a population where a subgroup has been randomized to receive an intervention that significantly reduces the incidence of primary RSV disease in infancy. Such an opportunity is provided by a randomized, double blinded, phase three clinical trial of a next generation RSV monoclonal antibody (motavizumab) that showed high efficacy for the prevention of RSV-associated medically attended lower respiratory illness (RSV-MALRI) events in a cohort of healthy, full term infants [140]. In this study, participants were followed through two subsequent winter RSV seasons following the first RSV season in which they were randomized to receive prophylaxis. With each medically attended lower respiratory illness (MALRI) event, nasopharyngeal secretions were collected. Here we assess whether infants randomized to receive motavizumab in their first winter RSV season (thus delaying their primary RSV illness) experienced an increase in RSV-MALRI frequency or severity in their second winter RSV season, compared to participants in the placebo group.

Methods

Study population

The full methods of the phase 3 double-blinded placebo controlled randomized trial of motavizumab (the parent study) have been published elsewhere [140]. Briefly, healthy Native American infants living on the Navajo Nation, White Mountain Apache and San Carlos Apache Indian reservations who were born at full-term (≥36 weeks gestation) and were less than 6 months of age at the time of enrollment, were randomized to receive either motavizumab or placebo during the winter RSV season (5 monthly doses, 2:1 randomization). Four cohorts of infants were enrolled over four consecutive RSV seasons between 2004 and 2009, for a total of 2127 participants. The current sub-study excluded the San Carlos Apache reservation participants, bringing the total number of participants to 2088 (1,392 participants randomized to motavizumab, 696 randomized to placebo).

Evaluation of medically attended lower respiratory tract illness (MALRI)

Study participants were followed from the time of study enrollment through three years of age and assessed for inpatient and outpatient medically attended lower respiratory tract illness. Lower respiratory tract illness events were reviewed for inclusion by study investigators and were defined as a medical diagnosis of bronchiolitis or pneumonia. In the absence of such a medical diagnosis, the occurrence of the lower respiratory illness was determined by the study investigator's review of the medical records for the presence of lower respiratory signs and symptoms including cough, retractions, ronchi, wheezing, crackles or rales, as well as associated signs or symptoms including coryza, fever and apnea.

Nasopharyngeal secretions collected within five days of the MALRI event date (hospital admission for inpatient events; doctor visit date for outpatient events) were considered to be within the analytic window and were included in the analysis. A nasopharyngeal specimen was collected at every MALRI visit. The collection of nasal wash secretions involved instilling 15 – 20 cc of Ringer's lactate solution into each nostril of a seated child with a bulb syringe and collecting it from the opposite nostril. In children who could not have a nasal wash specimen collected, a nasal aspirate was obtained by instilling 3 – 6 cc of sterile saline into the nose and withdrawing nasal mucus using a feeding tube with a suction device. One milliliter of nasopharyngeal specimen was mixed with 6 ml viral transport medium and then divided into 4 – 8 aliquots which were snap frozen immediately using liquid nitrogen or an ethanol/dry ice bath, and stored at -70°C. At facilities where snap freezing was not possible, aliquots were immediately stored at -80°C. After freezing, aliquots were shipped to central laboratories for storage. Those collected within 150 days of randomization (the RSV season) were tested for RSV A and B by PCR assay for the primary efficacy analysis. Aliquots of untested specimen remained in storage at -80°C with continuous temperature monitoring.

Definition of the second RSV season and selection of samples

We defined the 'RSV season' as the continuous time period between the middle of October of one year through the end of May of the following year. This definition is consistent with the enrollment and follow up periods for the motavizumab parent study, where children were enrolled between October 15th and December 31st, and were followed for medically attended RSV illness for 150 days following enrollment, with the

end of 150 day follow up falling between March 16th and May 30th, depending on the participant's enrollment date. For all participants in this analysis, the second RSV season was defined as the period of time between October 15th and May 30th in the calendar year(s) following study enrollment (Table 7.1). Specimens eligible for testing were those collected within five days of an inpatient or outpatient event that occurred during the second RSV season and which was determined to be a lower respiratory illness.

Specimen testing for this sub-study

For the present sub-study, stored nasopharyngeal secretions from events that occurred during the second RSV season were tested for RSV A and RSV B by a real-time PCR multiplex viral panel. For the multiplex panel, 350ul of nasal sample was extracted using the NucliSENS EasyMag kit (bioMerieux, Marcy l'Etoile, France) with an RNA eluate volume of 25ul. 10ul of eluate was used for the real-time NxTAG® Respiratory Pathogen Panel (Luminex Corporation, Austin, Texas).

Statistical Analyses

Statistical analyses were performed using STATA13 (StataCorp. 2013. College Station, TX). 2,088 participants provided 81% power (assuming a two-sided alpha of 0.05) to show increased risk of RSV hospitalization in the motavizumab group assuming a 8% RSV hospitalization rate in the placebo group and a 12% RSV hospitalization rate in the motavizumab group (a 50% increase). The intention to treat analysis included all participants who were enrolled in the parent study, with the exception of participants at

one study site for whom IRB approval for this sub-study was not sought. The per-protocol analysis included all participants who received all five doses of the study drug in their first RSV season and who remained in the study at the beginning of the second RSV season. The proportion of study participants with the study endpoints were compared with the Fisher exact test, with exact CI calculated for the relative risk. Chi-square and Student's t-tests were used for pairwise comparisons. P-values <0.05 were considered significant. A sensitivity analysis was conducted to take into account participants who had a respiratory event with no RSV test result available, as well as those who were lost to follow up.

Ethical Approval

Informed consent for participation in this study was obtained from a parent or guardian of participants. Approval for this study was obtained from the Johns Hopkins Bloomberg School of Health IRB, the Phoenix Area Indian Health Service IRB, and the Navajo Nation IRB.

Results

Out of the 2,088 participants enrolled in the motavizumab trial, 1,876 (89.8%) remained in the study at the start of the second RSV season, while the remaining 212 (10.2%) were lost to follow up. Loss to follow up did not differ by treatment group (motavizumab: 142/1,392 (10.2%); placebo: 70/696 (10.1%), p=0.92**).**

The mean age of participants who remained in the study at the beginning of the second RSV season was 11.6 months (range 9.5 months – 15.5 months). Approximately one third of the participants (34.1% [640 / 1,876]) experienced at least one MALRI event during the second season period, with 7.7% (144/1,876) experiencing at least one inpatient MALRI and 30.0% (561/1,876) experiencing at least one outpatient MALRI. Some participants experienced multiple events (Figure 7.1). When calculating and comparing rates of illness between treatment groups, we considered only the first event. Rates of all-cause inpatient MALRI were lower for both treatment groups in the second compared to the first RSV seasons (Table 7.2). Rates of all-cause outpatient MALRI were the same in both seasons for the placebo group, but higher in the second compared to the first season for the motavizumab group (Table 7.2). For both the motavizumab and placebo treatment groups, the proportion of MALRI events that were inpatient events was lower in the second RSV season compared to the first RSV season (Table 7.2). The mean age at inpatient MALRI events during the second RSV season (16.4 months) was not different than the mean age at outpatient events (16.1 months, p=0.21).

A total of 894 MALRI events occurred during the second season, with hospitalizations accounting for 17.2% (155/894) and outpatient events accounting for 82.7% (739/894). All of the stored nasopharyngeal specimens from MALRI events that were collected within the analytic window and could be located were tested for RSV, with 120/155 (77.4%) of all inpatient MALRI events and 480/894 (54.0%) of all outpatient events having a specimen tested (Figure 7.2). There was no difference in mean age at event or in

treatment group assignment for samples that were located and tested compared to those that were not (Table 7.3).

Of the 600 events with samples tested in the second RSV season, RSV was detected in 162 (27%), and was more frequently detected in inpatient compared to outpatient events (43/120 (35.8%) vs. 119/480 (24.8%)) respectively, p=0.02). RSV was detected November through May of the second season, with peak frequency occurring between January and March, when the mean age of participants was 14.6 months – 16.6 months (Figure 7.3). There was no difference in mean age for participants with inpatient RSV MALRI (16.5 months) compared to outpatient RSV MALRI (16.6 months, p=0.73). There was also no difference in age for participants with inpatient RSV MALRI (16.5 months) compared to those with inpatient non-RSV MALRI (16.2 months, p=0.61), but participants with outpatient RSV MALRI were older than those with outpatient non-RSV MALRI (16.6 months vs. 15.7 months, p<0.001). Participants with RSV MALRI in the first season had a (non-significant) reduction in rates of RSV MALRI in the second season, but an increase in rates of non-RSV MALRI in the second season, compared to those without RSV MALRI in the first season (Table 7.4).

As previously reported, the efficacy for motavizumab in the first RSV season was 87% for the prevention of inpatient RSV MALRI and 71% for the prevention of outpatient RSV MALRI [140]. We observed no statistically significant difference by treatment group in the proportion of participants with at least one MALRI, RSV-MALRI, or non-RSV-MALRI event in the second RSV season (Table 7.5). The results were consistent in

the per protocol analysis (Table 7.6). In a sensitivity analysis of the ITT population, there remained no difference in RSV inpatient or outpatient event rates in the second RSV season by treatment group when assuming frequency of RSV detection was the same in tested as in untested specimens, or when assuming all untested specimens were RSV-positive (Supplemental Tables 7.1 and 7.2). The same was true for a sensitivity analysis of participants lost to follow up (Supplemental Table 7.3). Within the MALRI events with a specimen collected and tested for RSV, there was a trend of increased detection of RSV in the motavizumab treatment group compared to the placebo group for both inpatient and outpatient MALRI events, though this did not reach statistical significance (Table 7.7, Figure 7.4). In the motavizumab group 27.0% (30/111) of second season RSV-MALRI events were inpatient events, compared to 25.5% (13/51) in the placebo group (p=0.84).

Discussion

It has been reported previously that motavizumab was highly effective for preventing RSV-associated medically attended illness compared to placebo when administered to healthy term infants during the first RSV season of life [140]. In the current study, we observed no significant difference in rates of medically attended RSV-illness, either inpatient or outpatient or both, by treatment group in the second RSV season of life. Similarly, we observed no difference in RSV severity (measured as the proportion of RSV illness events that resulted in hospitalization) by treatment group. These findings are consistent with an observational study of RSV transmission in early childhood, which

found age to be a greater predictor of severe illness with RSV infection than the primary nature of the infection [38].

Interestingly, we observed that compared to those without RSV MALRI in the first season, participants who experienced RSV MALRI in the first RSV season were less likely to have an RSV MALRI illness in the second RSV season but more likely to have a non-RSV MALRI during that time period. It may be that children who experienced RSV MALRI in the first season had increased risk factors for respiratory illness in the form of environmental exposures or host characteristics that persisted through the second season. Their experience of an RSV illness in the first season may have offered some immune protection from severe illness upon secondary infection, while they remained at increased risk for respiratory illnesses from other pathogens.

A limitation of this study is that we were only able to test samples from 77% of inpatient events and 54% of outpatient events that occurred in the second RSV season. The proportion of events without available samples did not differ by treatment group, so it is unlikely that missing data introduced bias into our analysis of risk of RSV MALRI by treatment group. However, only tested samples could contribute to the numerator events which were used to calculate MALRI rates in the second season, which could have exaggerated the relative reduction in second compared to first season events that we observed. The sample size for the parent study was calculated for children experiencing RSV MALRI events in the first year of life, when risk of respiratory hospitalizations is highest. A limitation of this study was the constrained statistical power to detect a

difference in RSV MALRI rates between the two treatment groups given the reduced rate of respiratory hospitalizations in the second year of life. Loss to follow up between enrollment and the start of the second RSV season, as well as incomplete specimen collection, further reduced statistical power. Awareness that the primary objective of the parent study was to assess motavizumab efficacy in the first RSV season may have reduced study staff prioritization to collect NP specimens from children in the second year of life, evidenced by specimen collection in 75-95% of MALRI events in the first season and in 71-83% of MALRI events in the second season. We assume that all MALRI events occurring in the second RSV season were captured, as all participants enrolled in the study use the Indian Health Service facilities that served as our study sites. It is possible that some events occurred while children were out of the area, but we do not expect that this would have differed by treatment group, and therefore should not have biased our inferences.

Overall, our results provide reassurance of no significant increase in RSV disease in the second RSV season following immunoprophylaxis in the first. We did, however, observe a non-significant 9% increase in RSV MALRI rates in the second RSV season for the motavizumab treatment group compared to the placebo treatment group, which was consistent in both the ITT and PPP analyses. We also observed a non-significant increase in the proportion of specimens from motavizumab group participants with RSV detected, compared to placebo participants. It may be that the increased frequency of primary RSV infections in the second RSV season results in a greater number of lower respiratory illness episodes, which in turns results in more medically attended events among the

motavizumab recipients that are RSV associated. However, if such an effect exists, it is of a small enough magnitude that our study lacked the power to detect it conclusively. Furthermore, if the small relative increase in rates of RSV MALRI that we observed for the motavizumab group truly does exist, it would correspond to a very small (<1%) absolute increase in RSV MALRI in the second season. Overall, we have shown that delaying primary RSV-illness beyond the most vulnerable period of young infancy significantly decreased the burden of medically attended RSV lower respiratory illness in our study population from birth through two years of age. These findings support the case for the development of RSV vaccines and immunoprophylaxis products to prevent RSV-illness in healthy full term infants, even with protection that is limited to the first RSV season.

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Tables and Figures

Table 7.1 Timing of first and second RSV seasons for the four enrollment cohorts in the motavizumab trial

		RSV Season 1		RSV Season 2			
		Start Study day 0	End Study day 150	Start	Study day	End	Study day
Enrollment Cohort 1							
	Enrollment Start[1]	11/15/04	4/14/05	10/15/05	334	5/30/06	561
	Enrollment End[2]	12/30/04	5/29/05	10/15/05	289	5/30/06	516
Enrollment Cohort 2							
	Enrollment Start	10/17/05	3/16/06	10/15/06	363	5/30/07	590
	Enrollment End	12/30/05	5/29/06	10/15/05	289	5/30/07	516
Enrollment Cohort 3							
	Enrollment Start	11/30/06	4/29/07	10/15/07	319	5/30/08	547
	Enrollment End	12/31/06	5/30/07	10/15/07	288	5/30/08	516
Enrollment Cohort 4							
	Enrollment Start	10/15/07	3/13/08	10/15/08	366	5/30/09	593
	Enrollment End	12/31/07	5/29/08	10/15/08	289	5/30/09	516

[1]The first date of enrollment of a participant in this cohort
[2]The last date of enrollment of a participant in this cohort

Figure 7.1 Number of all-cause medically attended lower respiratory illness (MALRI) events during the second RSV season, among participants with at least one MALRI event

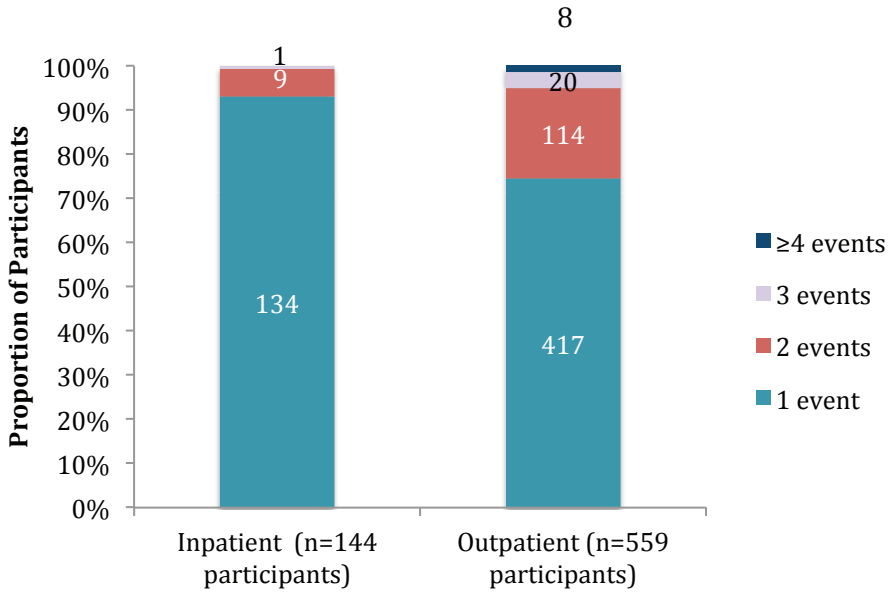


Table 7.2 Rates of medically attended lower respiratory illness events in the first and second RSV seasons, by treatment group

	RSV Season 1	RSV Season 2	p-value
Motavizumab Group	n=1,392	n=1,250[1]	
Any MALRI	390 (28.0%)	423 (33.8%)	**<0.01**
Inpatient MALRI	115 (8.3%)	98 (7.8%)	0.72
Outpatient MALRI	314 (22.6%)	368 (29.4%)	**<0.001**
Placebo Group	n=696	n=626[1]	
Any MALRI	278 (39.9%)	217 (34.7%)	0.05
Inpatient MALRI	116 (16.7%)	46 (7.4%)	**<0.001**
Outpatient MALRI	197 (28.3%)	193 (30.8%)	0.32
Proportion of all MALRI events that were inpatient			
Motavizumab	144/540 (26.7%)	104/592 (17.6%)	**<0.001**
Placebo	132/389 (33.9%)	155/894 (17.3%)	**<0.001**

[1]Excludes participants lost to follow up prior to the start of the second RSV season

Figure 7.2 Medically attended lower respiratory illness events in the second RSV season, with nasopharyngeal specimens collected and tested for RSV

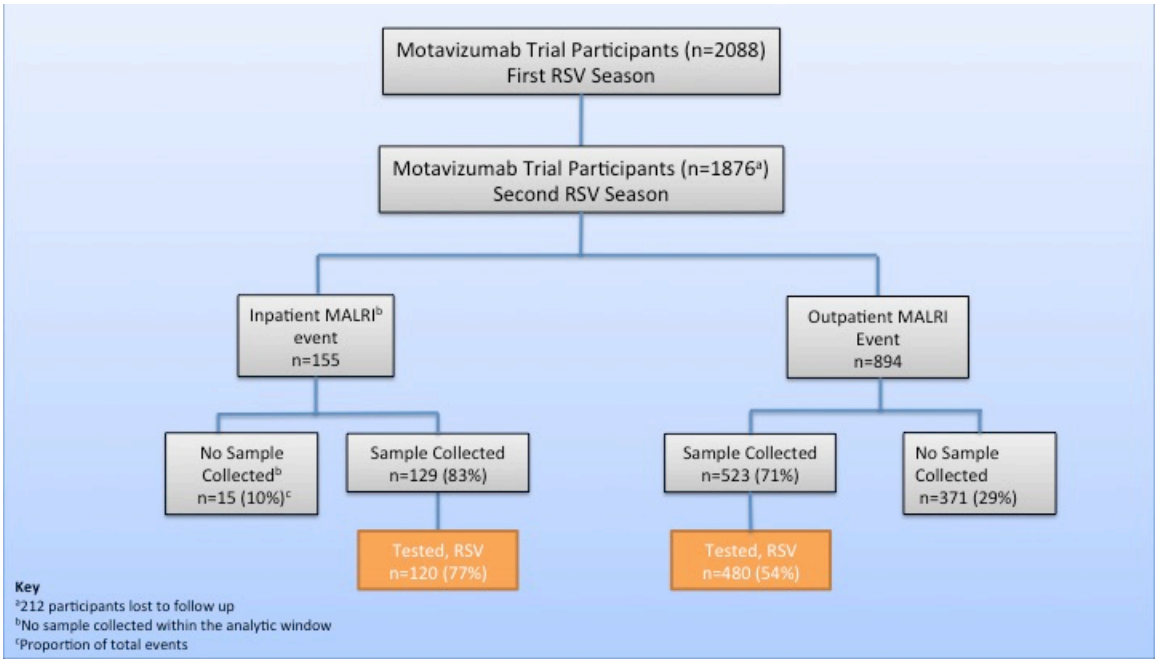


Table 7.3 Medically attended lower respiratory illness events occurring during the second RSV season with nasopharyngeal samples collected and tested for RSV

		Age		Treatment Group		
		Mean age, mo (SD)	**p- value[1]**	**Mota n (%)**	**Placebo n (%)**	**p-value[2]**
Inpatient				**n= 85**	**n= 44**	
	Tested (n=120)	16.3 (2.7)	0.62	78 (91.8)	42 (95.5)	0.44
	Not Tested (n=9)	16.8 (3.2)		7 (8.2)	2 (4.6)	
Outpatient				**n=344**	**n=180**	
	Tested (n=480)	15.9 (2.7)	0.06	313 (91.0)	167 (92.8)	0.48
	Not Tested (n=44)	15.1 (3.1)		31 (9.0)	13 (7.2)	
All Events				**n=429**	**n=224**	
	Tested (n=600)	16.0 (2.7)	0.13	391 (91.1)	209 (93.3)	0.34
	Not Tested (n=53)	15.4 (3.1)		38 (8.9)	15 (6.7)	

[1]Student's t-test
[2] χ^2 test

Figure 7.3 Frequency of inpatient and outpatient medically attended lower respiratory illnesses events by RSV season

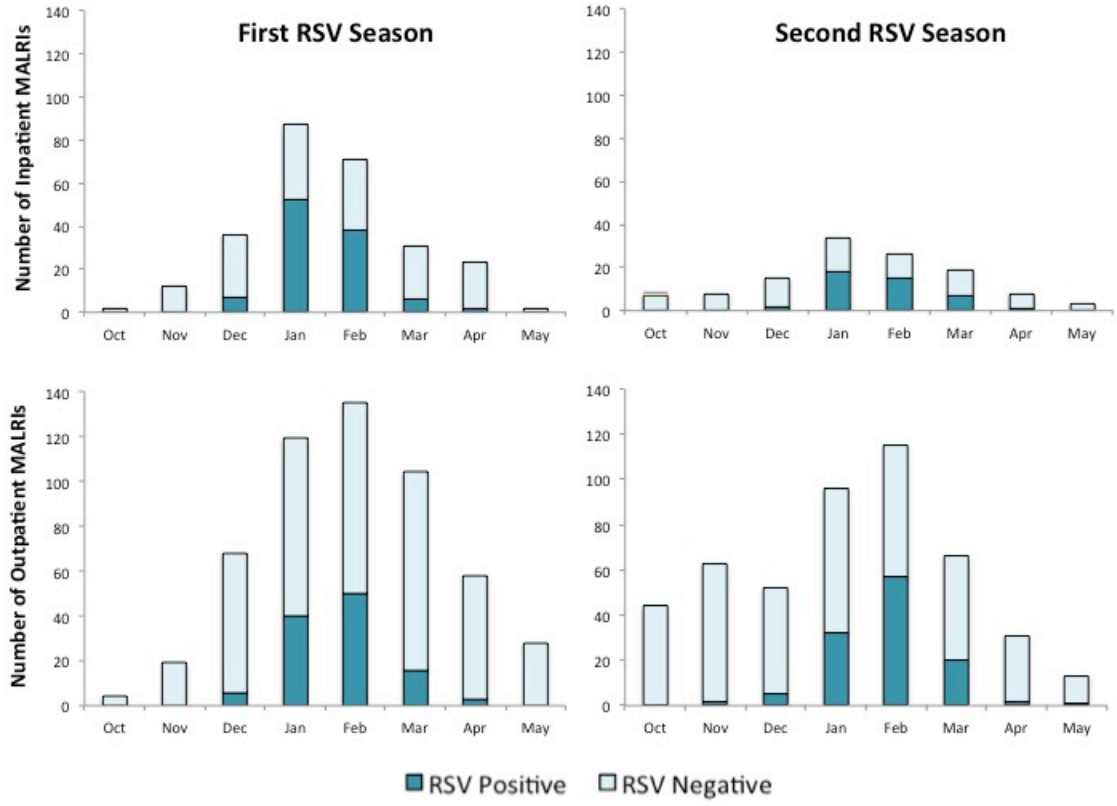


Table 7.4 Rates of RSV medically attended respiratory illness in the second RSV season, by RSV status in the first season[1]

	Any MALRI in second season[2]	Relative Risk (95% CI)	p-value	RSV MALRI in second season[3]	Relative Risk (95% CI)	p-value	Non RSV MALRI in second [3]season	Relative Risk (95% CI)	p-value
RSV inpatient event in first season (n=95)	41 (43.2%)	1.28 (1.01, 1.63)	0.06	5 (3.1%)	0.62 (0.26, 1.50)	0.28	27 (26.7%)	1.31 (0.94, 1.83)	0.13
No RSV inpatient event in first season (n=1,781)	599 (33.6%)			96 (5.0%)			405 (20.4%)		
RSV outpatient event in first season (n=104)	43 (41.3%)	**1**.23 (0.97, 1.56)	0.11	6 (5.5%)	0.70 (0.32, 1.54)	0.37	34 (31.2%)	**1.55 (1.16, 2.08)**	**<0.01**
No RSV outpatient event in first season (n=1,772)	597 (33.7%)			156 (7.9%)			398 (20.1%)		
RSV MALRI in first season (n=198)	84 (42.4%)	**1.28 (1.07, 1.53)**	**0.01**	11 (5.6%)	0.62 (0.34, 1.12)	0.10	61 (30.8%)	**1.39 (1.11, 1.75)**	**<0.01**
No RSV MALRI in first season (n=1,876)	556 (33.1%)			151 (9.0%)			371 (22.1%)		

[1]Restricted to participants not lost to follow up at the beginning of season 2
[2]Includes participants for whom a specimen was not collected at MALRI event occurring in second season, or collected but not available for RSV testing
[3]Numerators in this category are drawn only from the subgroup of 77% of inpatient events and 65% of outpatient events in the second season with samples collected and tested.

Table 7.5 Rates of medically attended lower respiratory illness in the second RSV season, by treatment group (intention to treat analysis)

	Motavizumab (n=1,250) N (%)	Placebo (n=626) N (%)	Relative risk (95% CI)	p-value	Absolute rate reduction, cases per 100 children (95%CI)
All-cause					
Inpatient MALRI	98 (7.0)	46 (6.6)	1.06 (0.76, 1.49)	0.71	-0.4 (-2.7, 1.9)
Outpatient MALRI	368 (26.4)	193 (27.7)	0.95 (0.82, 1.11)	0.53	1.3 (-2.8, 5.3)
Any MALRI	423 (30.4)	217 (31.1)	0.97 (0.85, 1.11)	0.71	0.8 (-3.4, 5.0)
RSV-associated[1]					
Inpatient RSV MALRI	30 (2.4)	13 (2.1)	1.16 (0.61, 2.20)	0.66	-0.3 (-1.7, 1.1)
Outpatient RSV MALRI	81 (6.5)	38 (6.1)	1.07 (0.73, 1.55)	0.73	-0.4 (-2.7, 1.9)
Any RSV MALRI	111 (8.9)	51 (8.1)	1.09 (0.79, 1.50)	0.59	-0.7 (-3.4, 1.9)
Non RSV-associated[1]					
Non-RSV Inpatient MALRI	47 (3.8)	27 (4.3)	0.87 (0.55, 1.39)	0.72	0.5 (-1.2, 2.2)
Non-RSV Outpatient RSV MALRI	255 (18.3)	132 (19.0)	0.95 (0.80, 1.13)	0.57	0.6 (-2.9, 4.2)
Any RSV MALRI	283 (20.3)	149 (21.4)	0.95 (0.80, 1.13)	0.57	1.1 (-2.6, 4.8)

[1]Numerators in this category are drawn only from the subgroup of 77% of inpatient events and 65% of outpatient events in the second season with samples collected and tested.

Table 7.6 Rates of medically attended lower respiratory illness in the second RSV season, by treatment group (per protocol analysis)

	Motavizumab (n=1,102) N (%)	Placebo (n=550) N (%)	Relative risk (95% CI)	p-value	Absolute rate reduction, cases per 100 children (95%CI)
All-cause					
Inpatient MALRI	92 (8.3)	42 (7.6)	1.09 (0.77, 1.55)	0.70	-0.7 (-3.5, 2.0)
Outpatient MALRI	336 (30.5)	171 (31.1)	0.98 (0.84, 1.14)	0.82	0.6 (-4.1, 5.3)
Any MALRI	388 (35.2)	193 (35.1)	1.00 (0.87, 1.15)	1.00	0.1 (-5.0, 4.8)
RSV-associated[1]					
Inpatient RSV MALRI	28 (2.5)	13 (2.4)	1.07 (0.56, 2.06)	1.00	-0.1 (-1.8, 1.4)
Outpatient RSV MALRI	77 (7.0)	32 (5.8)	1.20 (0.81, 1.79)	0.40	-1.1 (-3.6, 1.3)
Any RSV MALRI	105 (9.5)	45 (8.2)	1.16 (0.83, 1.63)	0.41	-1.3 (-4.2, 1.5)
Non RSV-associated[1]					
Non-RSV Inpatient MALRI	46 (4.2)	25 (4.5)	0.92 (0.57, 1.49)	0.70	0.4 (-1.7, 2.5)
Non-RSV Outpatient RSV MALRI	234 (21.2)	121 (22.0)	0.97 (0.97, 1.17)	0.75	0.7 (-3.5, 5.0)
Any RSV MALRI	261 (23.7)	136 (24.7)	0.96 (0.80, 1.15)	0.67	1.0 (-3.4, 5.4)

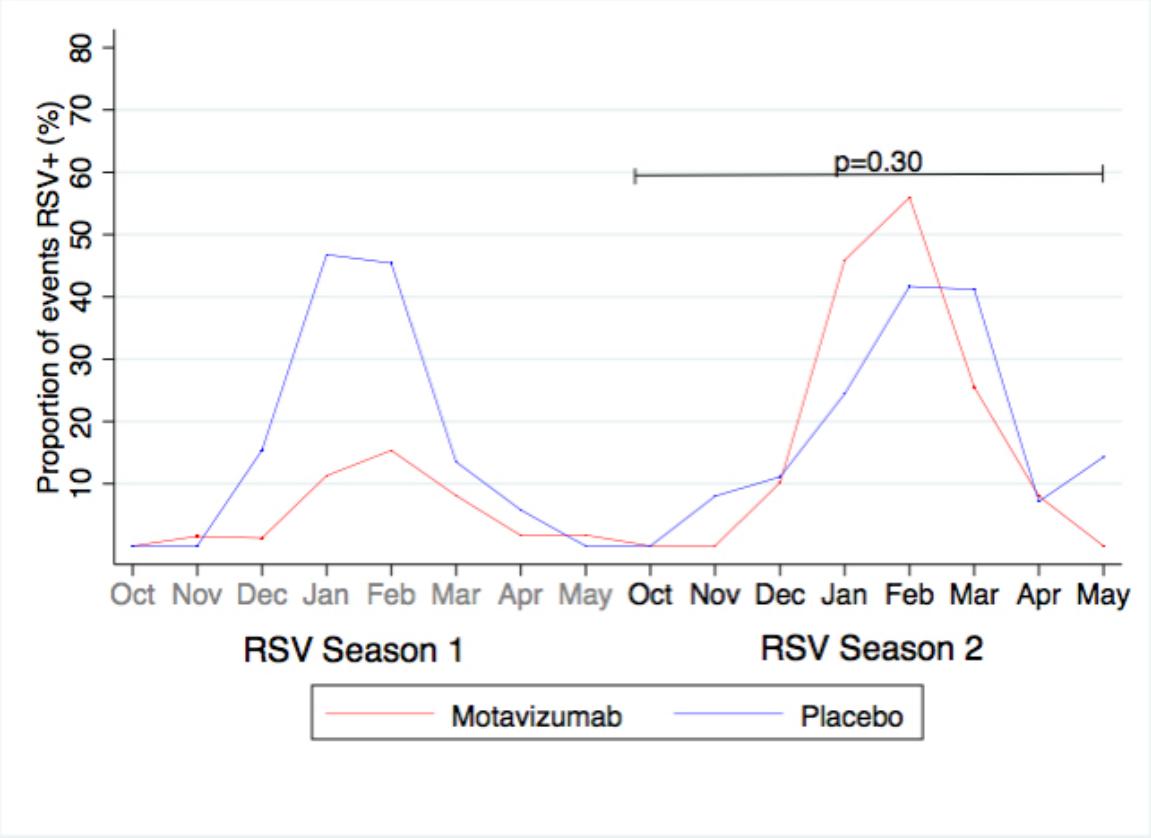
[1]Numerators in this category are drawn only from the subgroup of 77% of inpatient events and 65% of outpatient events in the second season with samples collected and tested.

Table 7.7 RSV prevalence by treatment group, among MALRI events with specimens tested[1]

MALRI Event Type	Treatment Group	RSV positive N (%)	p-value[2]
Inpatient			
	Motavizumab (n=78)	30 (38.5)	0.41
	Placebo (n=42)	13 (31.0)	
Outpatient			
	Motavizumab (n=313)	81 (25.9)	0.45
	Placebo (n=167)	38 (22.8)	
Any Event			
	Motavizumab (n=391)	111 (28.4)	0.30
	Placebo (n=209)	51 (24.4)	

[1]Denominators include events with specimen collected and tested for RSV
[2]χ^2 test

Figure 7.4 Proportion of MALRI events testing positive for RSV[1] by RSV season and treatment group



[1]Out of events with NP samples collected and tested

Supplemental Tables and Figures

Supplemental Table 7.1 Sensitivity analysis for events with missing RSV result for inpatient medically attended lower respiratory illness during second RSV season

	Motavizumab (n=1,392) n (%)	Placebo (n=696) n (%)	Relative Risk (95%CI)[1]	p-value[2]
Number of participants with RSV inpatient event	30 (2.2%)	13 (1.9%)	1.07 (0.56, 2.06)	1.00
Number of participants with inpatient event, with sample tested	74	37	-	-
Number of participants with inpatient event, with no sample tested	24	9	-	-
Number of additional hospitalizations based on rate #1[3]	10	3	-	-
Number of participants with RSV inpatient event, based on rate #2	40 (2.9%)	16 (2.3%)	1.25 (0.71, 2.22)	0.48
Number of additional hospitalizations based on rate #2[4]	24	9	-	-
Number of participants with RSV inpatient event, based on rate #2	54 (3.9%)	22 (3.2%)	1.23 (0.75, 2.00)	0.46

[1]Exact 95% confidence interval of relative risk
[2]p-value based on Fisher's exact test
[3]Rate 1:Assume proportion of RSV+ results is the same for tested as for untested specimens, within each treatment group (30/74 (40.5% positive) for motavizumab; 13/37 (35.1% positive) for placebo) and apply this proportion to untested specimens
[4]Rate 2: Assume all participants with a missing test result had an RSV+ result

Supplemental Table 7.2 Sensitivity analysis for events with missing RSV result for outpatient medically attended lower respiratory illness during second RSV season

	Motavizumab (n=1,392) n(%)	Placebo (n=696) n(%)	Relative Risk (95%CI)[1]	p-value[2]
Number of participants with RSV inpatient event	81 (5.8%)	38 (5.5%)	1.07 (0.73, 1.55)	0.77
Number of participants with inpatient event, with sample tested	225	117	-	-
Number of participants with inpatient event, with no sample tested	143	74	-	-
Number of additional hospitalizations based on rate #1[3]	51	24	-	-
Number of participants with RSV inpatient event, based on rate #2	132 (9.5%)	62 (8.9%)	1.06 (0.80, 1.42)	0.69
Number of additional hospitalizations based on rate #2[4]	143	74	-	-
Number of participants with RSV inpatient event, based on rate #2	224 (16.0%)	100 (14.4%)	1.12 (0.90, 1.39)	0.34

[1]Exact 95% Confidence interval of relative risk
[2]p-value based on Fisher's exact test
[3]Rate 1:Assume proportion of RSV+ results is the same for tested as for untested specimens, within each treatment group (81/225 (36.0% positive) for motavizumab; 38/117 (32.5% positive) for placebo) and apply this proportion to untested specimens
[4]Rate 2: Assume all participants with a missing test result had an RSV+ result

Supplemental Table 7.3 Sensitivity analysis for participants lost to follow up before the second RSV season

	Motavizumab	Placebo	Relative Risk (95%CI)	p-value[1]
Participants with MALRI[2] inpatient event in second season	98/1,250[3] (7.8%)	46/626[3] (7.3%)	1.07 (0.76, 1.49)	0.71
Participants with MALRI outpatient event in second season	368/1,250 (29.4%)	193/626 (30.8%)	0.95 (0.83, 1.10)	0.54
Number of participants lost to follow up before second RSV season	142	70	-	-
Number of additional hospitalizations based on rate #1[4]	11	5	-	-
Participants with inpatient event, based on rate #1	109/1,392[5] (7.8%)	62/696[5] (8.9%)	0.88 (0.65, 1.18)	0.40
Number of additional outpatient events based on rate #1	42	22	-	-
Participants with outpatient event, based on rate #1	410/1,392 (29.5%)	215/696 (30.9%)	0.95 (0.83, 1.09)	0.50
Number of additional hospitalizations based on rate #2[6]	142	70	-	-
Participants with inpatient event, based on rate #2	240/1,392 (17.2%)	116/696 (16.7%)	1.03 (0.85, 1.27)	0.74
Number of additional outpatient events based on rate #2	142	70	-	-
Participants with outpatient event, based on rate #2	510/1,392 (36.6%)	263/696 (37.8%)	0.98 (0.87, 1.11)	0.78
Participants with inpatient RSV MALRI second season	30/1,250 (2.4%)	13/696 (1.9%)	1.28 (0.67, 2.45)	0.44
Participants with outpatient RSV MALRI second season	81/1,250 (6.5%)	38/696 (5.5%)	1.17 (0.82, 1.73)	0.37
Number of additional RSV hospitalizations based on rate #3[7]	3	1	-	-
Participants with RSV inpatient event, based on rate #3	33/1,392 (2.4%)	14/696 (2.0%)	1.18 (0.63, 2.19)	0.60
Number of additional RSV outpatient events based on rate #3	9	4	-	-
Participants with RSV outpatient event, based on rate #3	90/1,392 (6.5%)	42/696 (6.0%)	1.07 (0.75, 1.53)	0.70
Number of RSV additional hospitalizations based on rate #4[8]	142	70	-	-
Participants with RSV inpatient event, based on rate #4	172/1,392 (12.4%)	83/696 (11.9%)	1.04 (0.81, 1.32)	0.78
Number of additional RSV outpatient events based on rate #4	142	70	-	-
Participants with RSV outpatient event, based on rate #4	223/1,392 (16.0%)	108/696 (15.%)	1.03 (0.84, 1.27)	0.77

[1]p-value based on Fisher's exact test
[2]Medically attended lower respiratory illness
[3]Denominator for participants remaining in study at the start of the second RSV season
[4]Rate 1: Assume proportion of participants lost to follow up (LTFU) with MALRI events is the same as for participants who remained in the study, and apply this proportion to those LTFU
[5]Denominator for all participants, including those LTFU
[6]Rate 2: Assume all participants lost to follow up had an inpatient and outpatient event
[7]Rate 3: Assume proportion of participants lost to follow up (LTFU) with RSV MALRI events is the same as for participants who remained in the study, and apply this proportion to those LTFU.
[8]Rate 4: Assume all participants lost to follow up had inpatient and outpatient RSV events

Chapter 8: Discussion

Summary of Study Findings

Interventions to prevent RSV disease in infancy, including vaccine candidates and an extended half-life monoclonal antibody, are rapidly advancing through clinical trials and may be considered for licensure within the next five to ten years [100]. Because the use of RSV immunoprophylaxis products has so far been restricted to high-risk infants, our understanding of how prevention of RSV disease in the general infant population may affect other acute respiratory illnesses and subsequent outcomes is limited. Drawing on the only clinical trial, to our knowledge, of an RSV immunoprophylaxis product in a healthy full term infant population, the findings from this thesis research will contribute to narrowing the gap in this evidence base.

8.1.1 Objective 1: To evaluate the role of RSV MALRI prevention on the prevalence and density of *Streptococcus pneumoniae* carriage in the infant nasopharynx

A growing evidence base supports the hypothesis that RSV and *S. pneumoniae* can interact synergistically to cause lower respiratory illness in children. Although *S. pneumoniae* carriage in the nasopharynx is common in childhood and does not usually result in lower respiratory illness, it is a required step for progression to pneumococcal disease. High nasopharyngeal colonization density is associated with pneumococcal pneumonia in children, which implies a role for the perturbation of pneumococcus in the nasopharynx on the progression to disease and the pathogenesis of pneumonia. The goal of this research objective was to assess whether preventing RSV lower respiratory illness

in infancy through prophylaxis with motavizumab was associated with reduced *S. pneumoniae* carriage in the nasopharynx, both in terms of overall prevalence and bacterial density. We hypothesized that the potential action of motavizumab against RSV in the upper respiratory tract could reduce interaction between these two pathogens in that space, thereby decreasing the presence and density of pneumococcus in the nasopharynx and reducing the risk of developing pneumococcal pneumonia in those with RSV MALRI compared to those without RSV MALRI. We also hypothesized that those prophylaxed with motavizumab could have reduced risk of pneumococcal pneumonia and that high pneumococcal density, which is a marker of pneumococcal pneumonia, would therefore be observed less frequently in the motavizumab treatment group. We found increased density, but not prevalence, of *S. pneumoniae* colonization of the nasopharynx in lower respiratory illnesses that were associated with RSV compared to those not associated with RSV. We observed a corresponding reduction in the density of pneumococcal carriage at medically attended lower respiratory illness events that occurred in the motavizumab treatment group compared to the placebo treatment group, although this did not reach statistical significance. We believe this provides further evidence that prevention of RSV lower respiratory tract disease may prevent LRI beyond RSV disease alone and provides a strong basis for the inclusion of pneumococcal outcomes in trials of RSV vaccines and additional monoclonal products.

8.1.2 Objective 2: To evaluate the impact of RSV MALRI prevention in infancy on MALRI with other respiratory viruses, and on subsequent medically attended wheezing at ages one to three years

Several observational studies have demonstrated an association between RSV illness in early infancy and an increased risk for subsequent wheeze or asthma. The motavizumab

trial, however, showed no difference by treatment group in rates of subsequent medically attended wheeze through 3 years of age, despite a significant reduction in RSV illness in infancy. The goal of this objective was to measure rates of medically attended lower respiratory illness associated with non-RSV viruses in children who were randomized to receive motavizumab or placebo throughout the RSV season, and to evaluate the independent contribution of RSV and other viruses to the risk of subsequent medically attended wheeze at ages one to three years. We hypothesized that some other, non-RSV, exposure was independently associated with risk of subsequent wheeze in this population, and that this might explain the lack of difference by treatment we observed in the subsequent wheezing outcomes. We detected viruses in approximately 90% of medically attended lower respiratory illnesses occurring during the winter season, with rhinovirus and RSV predominating. Motavizumab showed efficacy for preventing both lower respiratory illness with RSV alone and with RSV in combination with other viruses. For less severe illness (outpatient compared to inpatient events), however, motavizumab efficacy was reduced when other viruses were detected along with RSV compared to when RSV was detected alone. We believe this is evidence that some of the outpatient RSV associated events were attributable to other viruses with RSV either as a bystander or a co-infecting pathogen of the lower respiratory tract. The fact that the efficacy of motavizumab was equivalent for inpatient events with RSV alone or RSV in combination with other viruses supports a causal association of RSV for these more severe events. We did not find RSV illness in infancy to be independently associated with subsequent wheeze in this population, but did find that family history of asthma, exposure to other children who attend daycare, and medically attended respiratory illness with rhinovirus,

parainfluenza viruses, and coronaviruses in the first year of life were independently associated with subsequent medically attended wheeze between ages one and three. We also found that for the small group of infants who had an RSV hospitalization despite being prophylaxed with motavizumab, the risk of subsequent wheeze was significantly increased compared to placebo recipients who also had an RSV hospitalization, even after controlling for other risk factors. Our hypothesis is that there are a subset of children in the study population who have a host risk factor, or set of risk factors, that not only puts them at risk of serious illness when they have RSV infection, but that also increases their risk for subsequent wheeze in the future, independent of the RSV infection itself. Motavizumab is acting like a probe, revealing through prophylaxis failures a subgroup of children in the community who are inherently at high risk for wheezing.

8.1.3 Objective 3: To evaluate the risk of RSV MALRI in the second year of life following the prevention of RSV MALRI with motavizumab immunoprophylaxis in infancy

In the general population, infants less than five months of age are at highest risk for severe RSV disease, whereas older children tend to experience less severe illness with their RSV infections. The heightened risk of severe disease in young infants may be due to age-related factors including the immaturity of their immune systems and smaller bronchioles that are more easily obstructed. The increased risk of severe disease in this group may also be a consequence of the primary exposure to RSV, as re-infection has been shown in some studies to be less severe than the primary infection, though many of these studies are confounded by age. If a substantial fraction of the risk of severe RSV illness is attributed to the experience of the primary infection, regardless of age at which

it occurs, we would expect to see an increased incidence of severe RSV-illness in children whose primary infection is delayed beyond infancy, controlling for all other factors. The goal of this research objective was therefore to assess whether preventing RSV illness in infancy led to an increased risk of RSV associated medically attended lower respiratory illness in the second season of life in the motavizumab group compared to the placebo group. We found no statistical difference in rates of medically attended RSV illness, either inpatient or outpatient or both, by treatment group in the second RSV season, reassuring that there is not a substantial increased risk of medially attended respiratory events attributable to RSV in the second year of life among children who had protection against RSV disease as infants. We did observe a 9% relative increase in RSV MALRI in the second RSV season for the motavizumab compared to the placebo treatment group that was not statistically significant. This corresponded to an absolute rate increase of less than 1%. We also observed a trend of decreased severity of RSV MALRI in the second season compared to the first season for both treatment groups. The proportion of MALRI events with samples collected in the analytic window that were available for testing reduced our statistical power to detect true differences in rates of RSV MALRI between treatment groups in the second season. However, the small magnitude of the (non-statistically significant) increase in risk that we observed in the motavizumab group, in combination with less severe RSV MALRI in the second season provides strong support for the benefit of delaying primary RSV lower respiratory illness beyond infancy.

8.2 Implications for Policy and Practice

Our findings have implications for (1) the design of RSV vaccine and immunoprophylaxis efficacy trials and (2) for the investment case for such products.

An important finding from our research was the reduced efficacy we observed for outpatient RSV medically attended lower respiratory illness when other viruses are co-detected, compared to outpatient medically attended lower respiratory illness with RSV detected alone. Our finding implies that as the severity of RSV related disease increases, there is an increased likelihood that these are truly RSV attributable and therefore preventable by vaccine or monoclonal antibodies. For events that are less severe the finding of RSV has a lower likelihood, albeit still high, of causal attribution. This has particular implications for efficacy evaluations of vaccine and monoclonal antibody products that do not produce sterilizing immunity. While a substantial proportion of medically attended RSV disease burden occurs in outpatient settings, the fraction of lower respiratory illness in these settings that is causally associated with RSV is likely reduced compared to illness that warrants hospital admission. As the clinical severity threshold for RSV MALRI case definitions in efficacy trials is lowered, greater numbers of RSV-positive cases may be detected, but the product efficacy may be simultaneously driven down as the proportion of co-infections with respiratory viruses increases. This should be taken into consideration as case definitions for future trials are evaluated. It also provides a reason to evaluate respiratory secretions collected as part of RSV vaccine trials for the presence of other viruses.

Our research has potential implications for the investment case for future RSV vaccines and immunoprophylaxis products. Our findings suggest that there may be a role for RSV prevention in the reduction of transmission of *S. pneumoniae* and, by extension, a role for RSV vaccination in reducing the incidence of pneumococcal pneumonia in vaccinated children and in their communities through indirect protection. This warrants additional research (as described in section 8.4 below) and could be particularly relevant to settings that continue to have a high burden of bacterial pneumonia.

Our work also highlights the need for a better understanding of the role of RSV prevention on subsequent wheeze and childhood asthma so that the impact of vaccination on these outcomes can be more accurately predicted. Given the complexity of host/environment interactions in the development of asthma, it is possible that RSV-illness prevention in infancy will have a differential impact on incidence of subsequent wheeze and childhood asthma depending on the characteristics of the settings and populations where it is used. Finally, our work indicates no significant increase in risk of RSV associated lower respiratory illness in the second RSV season following immunoprophylaxis in the first, nor does it indicate significant viral replacement with motavizumab – an important consideration for vaccine introduction and one that has not been demonstrated previously in the context of RSV prevention in healthy infants.

8.3 Strengths and Limitations

A significant strength of this work was derived from it being embedded in a large, rigorously conducted double-blind randomized trial. We were fortunate to be able to

leverage the significant financial resources dedicated to generating the primary data and specimens, as well as the time required to conduct three years of follow up over four RSV seasons. From a methodological standpoint, the double-blinded randomized design of the motavizumab trial allowed us to evaluate associations within a study design that provides the strongest possible evidence of causation that can be achieved in epidemiologic studies. An additional advantage provided by the motavizumab trial was the opportunity to evaluate our research questions in a population of healthy full term infants. Given that this will be the likely target population for future licensed RSV vaccines, it is important to be able to generalize our findings to this group.

This research was also limited by some aspects of being embedded as part of a larger study. The parent study was designed to evaluate the efficacy of motavizumab for the prevention of (primarily inpatient) medical attended lower respiratory illness attributable to RSV. It was not designed to answer the questions we posed as part of this thesis work, and if we were designing studies *de novo* to address these research objectives we would have structured some aspects of them differently.

To better address the question of how RSV and *S. pneumoniae* interact synergistically in lower respiratory illness, we would have ideally included an outcome measure of pneumococcal pneumonia rather than nasopharyngeal colonization alone. Since this is a much rarer event than viral LRI, particularly in populations where PCV is used routinely, a significantly larger sample size would have been required to measure this association. Our study was also limited by only having access to nasopharyngeal samples. Serological

testing for RSV antibodies would also be beneficial to evaluate the presence of prior RSV infection in instances where the virus may have already been cleared from the nasopharynx. Pneumococcal colonization density can provide a useful proxy measure for risk of pneumococcal disease in children but a better study design to evaluate the association between colonization density and RSV infection would have included routine longitudinal sampling in order to assess whether preceding RSV infection predisposes to pneumococcal colonization, as hypothesized. In our investigation we also lacked pneumococcal serotype information, which could have provided additional insight into differences in colonization between those who were prophylaxed compared to those who were not.

All of our investigations relied on available specimens from the parent trial. In the first RSV season, respiratory events were less likely to have an available stored sample for testing if they came from the motavizumab group, or if they were RSV-negative. Because we measured rates of other viral illnesses in the first RSV season and only events with a sample collected could contribute to the numerators used in the rate calculations, there could have been differential misclassification bias whereby participants in the placebo group were more likely to be assigned as having an RSV or other viral lower respiratory illness compared to the motavizumab group. This could have led to an overestimation of the true efficacy of motavizumab for the prevention of viral lower respiratory illness. However, the sensitivity analysis that we conducted indicated that this was not likely the case, and the fact that we did not observe overall efficacy of motavizumab for prevention

of illness with non-RSV viruses provides some assurance that misclassification did not occur in a substantial number of participants.

The motavizumab trial design provided an ideal platform for the assessment of increased risk of RSV illness in the second RSV season, but because respiratory hospitalization rates significantly decrease in the second year of life, there was reduced statistical power to detect these second year events. This was compounded by a lower proportion of medically attended illnesses having nasopharyngeal samples collected, which further reduced our available sample size.

8.4 Future Research and Next Steps

Our findings highlight several areas where additional research would help to illuminate the questions we set out to address.

We observed an association between RSV-illness and increased pneumococcal colonization density, a finding that is consistent with several other studies, and that is also consistent with experimental evidence that RSV infection can facilitate pneumococcal virulence and adherence to host epithelial cells. Our study was the first, to our knowledge, to assess this relationship in the context of a randomized trial with an RSV immunoprophylaxis product and although we did not see a statistically significant decrease in pneumococcal colonization density among those prophylaxed, we saw a consistent trend in that direction. An extension of the current study would be to evaluate the distribution of pneumococcal serotypes detected among RSV-associated compared to

non-RSV respiratory events. At least one previous study has shown RSV infection to be associated with non-invasive pneumococcal serotypes, but the finding was only marginally statistically significant and the study population was restricted to children with radiographically confirmed pneumonia [251]. *S. pneumoniae* density has also been shown to vary by serotype [279], and the difference in colonization densities we observed between RSV-associated and non-RSV-associated events could in part be driven by differential serotype distribution between these two groups. Future studies are also needed to further investigate the role of RSV prevention on pneumococcal disease itself, rather than its proxy, pneumococcal carriage. In trials of RSV monoclonal antibodies and vaccines, proxy and direct measures of pneumococcal pneumonia such as chest radiographs and blood culture results should be included, when possible, in the clinical data that is collected so that the impact of RSV prevention on pneumococcal pneumonia can be assessed.

The finding of no difference in risk of wheeze by treatment group in the primary motavizumab trial analysis signaled that preventing RSV illness in early infancy may not result in reductions in subsequent wheeze at the population level. Our finding that medically attended respiratory illnesses with non-RSV viruses including rhinovirus, parainfluenza viruses and coronaviruses were independently associated with future medically attended wheeze, but RSV was not, suggest that in certain populations non-RSV viruses may play a larger role, causally or as a non-causal association, than RSV in the development of subsequent wheezing, and potentially childhood asthma. Further investigations of these associations are needed within the context of clinical trials. As part

of this research it will be important to follow children through an age where asthma can be diagnosed. A follow up of this cohort is underway now that the children are between 10 and 13 years of age.

The conflicting results observed between our study and studies of preterm infants with respect to RSV prevention and subsequent wheeze suggest that the role of RSV in the causal pathway to asthma may be different in the lungs of preterm infants compared to full-term infants, a potential area for future research. They also highlight the need to standardize subsequent wheeze outcome definitions going forward in order to allow for comparisons between studies.

Our findings support the notion that the development of childhood asthma is a complex phenomenon, involving a combination of genetic risk factors and environmental exposures, and that these may interact differently in the context of different phenotypes. In the subgroup of children who had RSV hospitalizations despite receiving motavizumab and who also had significantly increased risk of wheeze, we may have identified a particular high-risk phenotype for subsequent wheeze and potentially asthma. Future studies could be undertaken to better describe the host characteristics of such high-risk subgroups in the community.

Finally, although our research provides some reassurance that there is not significant increase in RSV disease in the second RSV season following immunoprophylaxis in the first, this question would be better addressed by studies with sample sizes that provide

greater statistical power to assess it. In settings where the severity of respiratory illness is reduced significantly in the second year of life it is unlikely that a small increase in rates of RSV illness following prophylaxis would lead to a meaningful increase in severe disease, but in some developing country settings the risk of elevated severe RSV illness remains high in the second year of life [46], and the risk of RSV in the second year of life following immunoprophylaxis in the first should be evaluated in these settings.

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Losonsky, G.A., Karron, R.A., Santosham, M. Efficacy of motavizumab for the prevention of RSV disease among healthy Native American infants. In press, **2015**.
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CURRICULUM VITAE

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PERSONAL DATA

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EDUCATION AND TRAINING

Expected January 2018	Doctor of Philosophy, International Health Global Disease Epidemiology and Control Johns Hopkins School of Public Health Baltimore, MD Advisor: Professor Katherine O'Brien Thesis project: *An evaluation of the role of respiratory syncytial virus, alone and with other pathogens, in causing respiratory disease among Native American children*
January 2007	Master of Health Sciences, International Health Global Disease Epidemiology and Control Johns Hopkins School of Public Health Baltimore, MD
May 2004	Bachelor of Sciences, Environmental Sciences College of Natural Resources University of California at Berkeley Berkeley, CA
August 2001 – June 2002	Study Abroad University of Ghana, Legon Accra, Ghana

PROFESSIONAL EXPERIENCE

Position:	Pre-doctoral Investigator
Institution:	PI: Professor Kate O'Brien and Professor Laura Hammitt, Center for American Indian Health Department of International Health Johns Hopkins Bloomberg School of Public Health Baltimore, MD 21205
Dates:	February 2015 - Current
Principal Responsibilities:	• Evaluate association between RSV prevention in infancy and carriage and density of *Streptococcus pneumoniae* in the infant nasopharynx • Evaluate efficacy of motavizumab immunoprophylaxis for prevention of RSV alone and in combination with other viral pathogens • Evaluate the independent effects of respiratory pathogens and baseline risk factors on risk of subsequent medically attended wheezing between ages one and three years • Evaluate the risk of increased risk of RSV disease in the second year of life following the prevention of RSV disease in infancy
Position:	Graduate student researcher The Pneumoniae Etiology Research for Child Health (PERCH) study
Institution:	PI: Professor Kate O'Brien, International Vaccine Access Center Department of International Health Johns Hopkins Bloomberg School of Public Health Baltimore, MD 21205
Dates:	September 2012 – Current
Principal Responsibilities:	• Evaluated effect of antibiotic exposure and blood culture volume on detection of

pathogens from children with severe and very severe pneumonia
- Evaluated microarray methods for pneumococcal serotyping
- Evaluated automated PCR reading software
- Described epidemiology of RSV associated pneumonia hospitalizations in the PERCH study
- Managed and reviewed study-wide laboratory results data
- Coordinated sub-study collaborations with external laboratories
- Coordinated specimen/isolate biorepository and manage sample inventories

Position:	Graduate student researcher Real-time Monitoring of Under-Five Mortality Project
Institution:	PI: Professor Agbessi Amadou The Institute for International Programs Department of International Health Johns Hopkins Bloomberg School of Public Health Baltimore, MD 21205
Dates:	August 2013 – December 2013

Principal Responsibilities:

- Supervised local field teams for 10 weeks in Northern Ghana for an end-line survey to assess the effectiveness of a community-based volunteer system for registering births and deaths in three rural districts.
- Trained interviewers in the use of a CSPro computer-based survey, assisted interview teams in the field every day throughout the 10-week survey, conducted interview quality assessments, monitored interview progress, liaised with Accra and Baltimore based investigators, and assessed data quality.

Position:	Project Coordinator, The Pneumoniae Etiology Research for Child Health (PERCH) study
Institution:	PI: Professor Kate O'Brien International Vaccine Access Center Department of International Health Johns Hopkins Bloomberg School of Public Health Baltimore, MD 21205

Principal responsibilities:

- Monitored protocol adherence and methods standardization at seven international research sites through site visits, monitoring of data quality indicators, and regular communications with field staff and site principal investigators
- Reviewed study results and study performance monitoring data in real time, prepared progress reports for internal and external stakeholders
- Managed cross-site laboratory and data management working groups to develop SOPs and data collection materials and to conduct ongoing training and troubleshooting
- Prepared sub-study proposals, assisted in analyses and manuscript preparation
- Coordinated development and IRB submission of a standardized clinical protocol for JHSPH research institutions affiliated with the project research sites
- Conducted and published a head-to-head evaluation of candidate molecular diagnostic platforms
- Developed quality indicators to monitor the performance of the study sites and to track study progress against objectives
- Created scopes of work and budgets for seven international research sites; generated a site initiation checklist and pilot report to be standardized across seven research sites
- Worked with consultants to create a model to illustrate the trade-offs between laboratory budget, sample size, and statistical power in the study

Position:	Laboratory and Clinical Coordinator The Rwanda Zambia HIV Research Project Lusaka, Zambia and Kigali, Rwanda
Institution:	PI: Susan Allen Department of Global Health Emory University Rollins School of Public Health Atlanta, GA 30322
Dates:	January 2007 - December 2008

Principal Responsibilities:

- Coordinated clinical operations for large observational studies; oversaw study participant enrollment and follow-up, clinic research operations, family planning services and quality control management of all clinical data maintained in study source documents, case report forms and medical charts
- Provided on-going consultation and support for the development, implementation, and evaluation of Good Clinical Practice (GCP); conducted oversight and management of human subject regulatory documents, including consent forms and subject clinical information for more than 2,000 study participants.
- Managed a central research laboratory and three satellite clinical laboratories based in Lusaka, Zambia and Kigali, Rwanda, ensuring continuous adherence to five study protocols and quality control of results for more than 3,000 study participants as well as personnel management, training, and periodic evaluation of 27 laboratory technicians and four specimen repository managers.
- Implemented and maintained Good Clinical Laboratory Practice (GCLP), prepared for audits and external evaluations resulting in full GCLP accreditation of Project San Francisco laboratories, the second African research facility outside of South Africa to achieve this performance standard.

- Reviewed and assisted in the development of laboratory analytical plans for observational studies and clinical trial protocols; trained laboratory staff and prepared facilities for participation in an IAVI-funded global multi-site Phase II HIV vaccine trial.

Position: Graduate Student Researcher
The Nepal Nutrition Intervention Project
Kathmandu, Nepal

Institution: PI: Joanne Katz
Department of International Health
Johns Hopkins Bloomberg School of Public Health
Baltimore, MD 21205

Dates: July 2006 – November 2006

Principal Responsibilities:

- Wrote a monograph summarizing maternal health research findings from two major community trials, sythesizing unpublished data as well as results from more than 15 publications based on research conducted between 1994 and 2002 as part of the NNIPS-2 and NNIPS-3 trials in Sarlahi, Nepal.
- Worked with field site staff to obtain hands-on training in the design and conduct of large-scale community trials.

Position: Research Assistant, The Schistosome Research Project

Institution: PI: Robert Spear
Center for Occupational and Environmental Health
University of California at Berkeley
Berkeley, CA 94720

Dates: June 2004 – July 2005

Principal Responsibilities:

- Conceived and optimized a novel PCR assay for the detection of *Schistosoma japonicum* cercariae; developed a laboratory protocol to demonstrate the assay's specificity and sensitivity and conducted field-testing in rural southwestern China in collaboration with field site staff.
- Developed new inter-disciplinary partnerships between the Environmental Engineering, Infectious Disease, and Environmental Health Sciences departments at UC Berkeley in support of the research objectives.

REFEREE / REVIEW EXPERIENCE:

- *Emerging Infectious Diseases Journal*
- *American Journal of Tropical Medicine and Hygiene*

PUBLICATIONS:

1. Higdon MM, Hammitt LL, Deloria Knoll M, Baggett HC, Brooks WA, Howie SRC, Kotloff KL, Levine OS, Madhi SA, Murdoch DR, Scott JAG, Thea DM, **Driscoll AJ**, Karron RA, Park DE, Prosperi C, Zeger SL, O'Brien KL, Feikin DR, The PERCH Study Group. Should controls with respiratory symptoms be excluded in case-control studies of pneumonia etiology? Reflections from the PERCH Study**.** Clin Infect Dis **2017** 64:S205-S212

2. Crawley J, Prosperi C, Baggett HC, Brooks WA, Deloria Knoll M, Hammitt LL, Howie SRC, Kotloff KL, Levine OS, Madhi SA, Murdoch DR, O'Brien KL, Thea DM, Awori JO, Bunthi C, DeLuca AN, **Driscoll AJ**, Ebruke BE, Goswami D, Higdon MM, Karron RA, Kazungu S, Kourouma N, Mackenzie G, Moore DP, Mudau A, Mwale M, Nahar K, Park DE, Piralam B, Seidenberg P, Sylla M, Feikin DR, Scott JAG, The PERCH Study Group. Standardization of clinical assessment and sample collection across all PERCH study sites. Clin Infect Dis **2017** 64:S228-S237

3. Watson NL, Prosperi C, **Driscoll AJ**, Higdon MM, Park DE, Sanza M, DeLuca AN, Awori JO, Goswami D, Hammond E, Hossain L, Johnson C, Kamau A, Kuwanda L, Moore DP, Neyzari O, Onwuchekwa U, Parker D, Sapchookul P, Seidenberg P, Shamsul A, Siazeele K, Srisaengchai P, Sylla M, Levine OS,

Murdoch DR, O'Brien KL, Wolff M, Deloria Knoll M. Data management and data quality in the Pneumonia Etiology Research for Child Health study, a large international case-control study of severe childhood pneumonia. Clin Infect Dis **2017** 64:S238-S244

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10. Baggett HC, Watson NL, Deloria Knoll M, Brooks WA, Feikin DR, Hammitt LL, Howie SRC, Kotloff KL, Levine OS, Madhi SA, Murdoch DR, Scott JAG, Thea DM, Antonio M, Awori JO, Baillie VL, DeLuca AN, **Driscoll AJ**, Duncan J, Ebruke BE, Goswami D, Higdon MM, Karron RA, Li M, Moore DP, Morpeth SC, Mulindwa JM, Park DE, Paveenkittiporn W, Piralam B, Prosperi C, Sow SO, Tapia M, Zaman K, Zeger SL, O'Brien KL, The PERCH Study Group. Density of upper respiratory colonization with *Streptococcus pneumoniae* and its role in the diagnosis of pneumococcal pneumonia among children aged <5 years in the PERCH Study. Clin Infect Dis **2017** 64:S317-S327

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determining pneumonia etiology in children. Clin. Infect. Dis., **2012**; Clin Infect Dis. (2012) 54(suppl 2): S146-S152

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30. **Driscoll, A.,** Kyle, J., and Remais, J. Development of a novel PCR assay capable of detecting a single Schistosoma japonicum cercaria recovered from Oncomelania hupensis. *Parasitology*. 2005 131:497-500

TEACHING EXPERIENCE

Johns Hopkins Bloomberg School of Public Health
Department of Epidemiology

2015, 2016, 2017	Power and Sample Size for the Design of Epidemiological Studies Primary Instructors: Xiangrong Kong and Stephen Gange Teaching assistant

Johns Hopkins Bloomberg School of Public Health
Department of International Health

2015, 2016 (220.601.01)	Foundations of International Health Primary Instructor: David Peters Lead teaching assistant

2015, 2016	Social and Behavioral Foundations of Primary Health Care Primary Instructor: Bill Brieger Teaching assistant
2014	Biological Basis of Vaccine Development Primary Instructors: Anna Durbin and Jay Bream Teaching assistant
2012, 2013	Special topics in vaccine science Teaching assistant

Johns Hopkins Bloomberg School of Public Health
Department of International Health
Center for American Indian Health Summer Institute

2012, 2016	Introduction to Data Management Using Native American Health Data Primary Instructor: Maria Knoll Teaching assistant (2016) Co-instructor (2012)
2014	Introduction to Quantitative and Qualitative Research Methods for American Indian Health Primary Instructors: Laura Hammitt and Mary Cwik Teaching assistant
2013	Collecting, Analyzing and Using Public Health Data in Native American Communities Teaching assistant

PRESENTATIONS

1. **Driscoll, A.J**., Hammitt, L., Weatherholtz, R., Moulton, L., Goklish, N., Reid, R., Santosham, M., Karron, R., O'Brien, K. Risk of wheeze among children ages 1-3 years following the prevention of medically attended RSV A/B illness with motavizumab monocolonal antibody. Accepted as a poster

presentation at the RSV-16 Symposium, September 2016, Patagones, Argentina.

2. **Driscoll, A.J.,** The PERCH Study Team, Jason Hinds and Katherine A. Gould. Serotyping of pneumococcus from STGG specimens for the PERCH study using direct and culture based microarray techniques. Accepted as an oral presentation at the International Symposium for Pneumococcus and Pneumococcal Disease, June 2016, Glasgow, Scotland.

3. **Driscoll, A.J.** on behalf of the PERCH study team. Factors affecting pneumococcal blood culture yield in children <5 years in with severe or very severe pneumonia in the Pneumonia Etiology Research for Child health (PERCH) Study. Accepted as a poster presentation at the International Symposium for Pneumoccocus and Pneumococcal Disease, March 2014, Hyderbad, India.

4. DeLuca A.N., **Driscoll, A.J**., Feikin, D.R., Knoll, M.D, Bhat, N.,Karron, R.A., Crawley, J., Scott, J.AG., Murdoch, D.R., O'Brien, K.L., Brooks, W.A., Howie, S.R., Kotloff, K.L., Madhi, S.A., Maloney, S.A., Thea, D.M., Levine, O.S. The Pneumonia Etiology Research for Child Health (PERCH) Project. Poster Presentation at the Children's Hospital of Philadelphia Fourth Annual Pediatric Global Health Symposium, 2011. Philadelphia.

5. Zurawski, V., **Driscoll, A.J.,** Deluca, A., Knoll, M., Murdoch, D., O'Connor, O., Dupont-Rouzeyrol, M., D'Ortenzio, E., Missotte, I., Moisi, J., Besson-Leaud, L., Chevalier, C., Debarnot, V., Levine, O., Mermond., S. Aetologies of lower respiratory tract infections in hospitalized children in New Caledonia: A PERCH pilot study. Poster presentation at the Annual Meeting of the International Network of Institute Pasteur, 2010. Hong Kong.

6. Mukamwezi, J., Mukankuku, M., Karita, E., Wong, F., Grabowski, M.K., Bayingana, R., **Driscoll, A.J**., Tichacek, A., Allen, S. The Effect of Couples' Counseling and Testing on Condom use among HIV discordant couples in Rwanda. Poster Presentation at the International AIDS Vaccine Initiative 3rd Interest Work Shop 2009. Lusaka, Zambia.

7. Kelley, A**.**, **Driscoll, A**.J., Karita, E. , Grabowski K., Allen, S. Seroprevalence of Hepatitis B and C infection within a cohort of HIV-discordant couples in Kigali, Rwanda: Implications for vaccine trial recruitment. Poster Presentation at The AIDS Vaccine Conference 2008. Cape Town, South Africa.

8. **Driscoll, A.J.,** PCR detection of *Schistosoma japonicum* cercariae targeting the SjR2 retrotransposon. Presentation at the Schistosome/Filaria Joint Genome Network meeting at the National Institutes of Health (NIH), Bethesda, Maryland 2004.